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S. C. J. van der Putte

The Development of the Lymphatic System in Man

With 33 Figures

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Dr. S. C. J. van der Putte
Institute of Pathology
University of Utrecht
Pasteurstraat 2, Utrecht
The Netherlands

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List of Abbreviations

acv	anterior cardinal vein
adr	adrenal gland
als	axillary lymph sac
aut	autonomic nervous tissue
av	azygos vein
baa	branchial arch artery
bp	brachial plexus of nerves
br	bronchus
bt	bifurcation of the trachea
cca	common carotid artery
ce	caudal extension of the jugulo-axillary lymph sac
cia	common iliac artery
cl	clavicula
cv	caudal vein
e	esophagus
eia	external iliac artery
eiv	external iliac vein
ejv	external jugular vein
hav	hemiazygos vein
icv	inferior caval vein
iiv	internal iliac vein
ijv	internal jugular vein
ilp	iliac lymph plexus
isb	intersubcardial anastomosis
itlp	internal thoracic lymph plexus
itv	internal thoracic vein
jals	jugulo-axillary lymph sac
jls	jugular lymph sac
lk	kidney
	lung
ll	lymphatic(s)
llp	lumbar lymph plexus
lv	lumbar vein
mlp	mesenteric lymph plexus
n	nerve
pa	pulmonary artery
pcc	pericardial cavity
pcv	posterior cardinal vein
ptc	peritoneal cavity
ptlp	paratracheal lymph plexus
puv	primitive ulnar vein
ra	renal artery
rv	renal vein
sacv	sacrocardinal vein
sbcv	subcardinal vein
sc	scapula
scla	subclavian artery
sclv	subclavian vein
scmm	sternocleidomastoid muscle
scv	superior caval vein
spcv	supracardinal vein
srv	suprarenal vein
stlp	subtracheal lymph plexus
t	trachea
td	thoracic duct
tm	trapezius muscle
v	vertebra
vv	vein(s), venule(s)
vn	vagus nerve
↓	lymphatico-venous communication

I. Introduction

When Budge in the eighth decade of the nineteenth century, started his investigations into the origin and development of the lymphatic system, he probably did not expect that these would be the starting-point for disputes that would continue well into our time. But rather than by his results were these disputes intensified by the controversial conclusions of some papers published by Gulland (1894), Ranvier (1897) and Sala (1900), and above all by some new investigations by Sabin (1902–1913) and Huntington (1908–1914) during the first decades of the twentieth century.

The most important controversial point was the difference in opinion about the origin of the lymphatic system. An extensive review of the relevant literature is found in O. F. Kampmeier's book: "Evolution and Comparative Morphology of the Lymphatic System"; Thomas, Springfield, U.S.A. (1969). Whereas some investigators were of the opinion that the lymphatic system was formed from the venous system, there were many others, who believed that this system originates from confluent spaces in the mesenchyme and secondarily communicates with the veins.

The most prominent advocate of the first mentioned opinion was Sabin (1902–1913). She believed that the lymphatics are formed by a process of sprouting from the large central veins in a limited number of definitely localized areas, but that these sprouts demonstrate almost immediately their own lymphatic character. From these sprouts separate primordia develop at first; these enlarge, confluence and form new sprouts which grow out into more peripheral parts of the embryo.

Both Lewis (1905) and Hoyer (1908, 1912, 1934) agreed with her on the venous derivation of the lymphatic system, but their opinions differed from hers in some other respects. Lewis believed that the early lymphatic primordia were formed by long series of isolated, but initially true venous, branches. Hoyer, although accepting Sabin's view concerning their lymphatic character from the beginning, was convinced more or less in accordance with Lewis that these primordia were more numerous than Sabin suggested.

Sabin's theory was strongly attacked by Huntington (1908–1914), McClure (1908–1915) and Kampmeier (1912–1969), who were of the opinion that the lymphatic system does not sprout from already existing veins, but develops independently from them in the mesenchyme. According to these investigators the lymphatics are formed more peripherally in the embryo by confluence of numerous spaces inside the mesenchymal tissue. These lymphatics grow centripetally by annexing other similar spaces. Finally, in certain places, anastomoses

are established with the venous system. The bordering mesenchymal cells become endothelial cells by flattening caused by increasing pressure.

Besides these two oppositional groups there were other, more individual investigators, who occupied a sort of central standpoint (Sala, 1900; van der Jagt, 1932; and Kutsuna, 1933). They believed that the lymphatic system originates both from the veins and from confluent spaces in the mesenchyme. According to them, small venules, isolated from the main venous channels at some specific places, enlarge by coalescence with spaces in the adjacent mesenchyme.

This controverse about the origin of the lymphatic system in general, is reflected in the conclusions drawn from investigations into human development.

Sabin (1909, 1913) discerned two stages in the early development of the human lymphatic system. During the first phase (embryos from 8 to 20 mm C–R length) the "jugular lymph sacs" develop bilaterally just lateral to the confluence of the anterior and posterior cardinal veins. At first, these rather small, sac-like vessels are often distended by blood cells, opening into the veins by small apertures. Within a short period they enlarge greatly, while the communications with the veins continue to exist. During the second phase other lymph sacs, the thoracic duct and the cisterna chyli develop. In the abdomen the retroperitoneal or mesenteric lymph sac was identified ventral to the aorta. It originated, she believed, from the intersubcardinal anastomosis, to extend mainly into the mesentery. Besides this primordium, another paired lymph structure was found, namely the posterior or iliac lymph sac. It is situated lateral to the aorta near the renal veins, from which it seems to have sprouted. Somewhat later, connections are established between these primordia, and by continuance into a cranial direction the cisterna chyli and the caudal part of the thoracic duct are formed. This caudal part meets a downward extension of the left jugular lymph sac, which accompanies the descending aorta from the left to the right side of the embryo. Thus a communication is established between the abdominal and the jugular lymphatics. From this "primary lymphatic system" many sprouts grow outward and invade more peripheral regions.

As mentioned above, Lewis (1909) agreed with Sabin in so far as the venous origin of the lymphatic system was concerned. From observations in the jugular region of human embryos of 10, 11.5, 16 and 22.8 mm C–R length he concluded, however, that the jugular lymph sac of each side is formed by fusion of more, probably up to four, primordia. Sometimes venous communications were observed, but this was not a constant feature. It must be mentioned however, that his assistant Thyng (1914) some years later, while reconstructing an 17.8 mm embryo, could easily detect openings between the large jugular lymph sacs and the veins at the confluence of the internal and external jugular veins. Besides these jugular lymph sacs however, Thyng came upon another lymph sac in this rather young embryo. This also was sac-like in form. It was filled with blood and situated ventral to the abdominal aorta between the superior and inferior mesenteric arteries. It seemed entirely isolated from the nearby intersubcardinal anastomosis. By position is was the mesenteric lymph sac, which apparently developed much earlier than Sabin believed.

In total disagreement with these investigators, Kampmeier (1931, 1960, 1969) was of the opinion that similar to the mode of development of the lymphatic system, as observed by him in some kinds of animals (Kampmeier, 1912, 1915,

1920, 1922, 1925, 1960), the human lymphatic system also was of mesenchymal origin. In human embryos he studied the origin and development of the jugular lymph sacs, the thoracic duct and the cisterna chyli. In the development of the jugular lymph sacs five phases were discerned. During the first phase (embryos from 9 to 12 mm) venous networks grow out from the proximal parts of the anterior cardinal veins into the lateral mesenchyme. These plexuses split up into some vessels, which drain the fore limb, and other sac-like structures often filled with blood, which during the second phase subsequently lose their connections with the anterior cardinal veins until at last only one opening, the most caudal, remains. Then, during the third phase, in the mesenchyme around these isolated veins, named by him "venolymphatics", numerous vacuoles appear, which enlarge and fuse with one another. During the next phase this process continues, true lymphatics being formed, which are entirely seperated from the venous system and contain in their lumina the remnants of the wall of the "venolymphatics". It is not until the embryos have reached a length of 30 mm that new and definitive lymphatico-venous communications are established at the jugulo-subclavian confluence. After that, the lymph sacs become filled up for the most part by the development of lymphoid tissue in walls and septa. The rest of the sacs form the jugular lymph truncs and the upper part of the thoracic duct (right lymphatic duct).

In embryos from 20 to 30 mm the thoracic ducts and the cisterna chyli develop in essentially the same way. The thoracic ducts develop bilaterally by confluence of vacuoles in the mesenchyme just outside degenerating and shringing veins dorsomedial to the anterior cardinal veins and ventral to the azygos and hemiazygos veins. More caudally, the cisterna chyli is formed in quite the same way around regressing parts of the supracardinal veins at the level of the kidneys. Kampmeier did not describe the origin of other parts of the abdominal lymphatic system.

According to Balankura (1951), their mode of origin is completely the same as described by Kampmeier as regards the cisterna chyli. Balankura rejected the idea of separate lymph sacs as given by Sabin.

From investigations into the origin and development of the vasa sanguinea vasorum of the wall of the descending aorta (van der Putte, 1969) some questions remained particularly about the origin and development of the lymph vessels of this region. At first, no problems were met, probably because embryos larger than 30 mm C-R length were involved. However by subsequently studying younger embryos, the observations were no longer in accordance with those reported in the relevant literature, i.e. Kampmeier's observations. Meanwhile, it became evident that our knowledge about the development of the human lymphatic system was far from complete and re-study of this particular problem seemed to be fully justified.

II. Material and Methods

In this investigation 40 human embryos between 8 mm total length and 33 mm C-R length (estimated ages between 35 and 65 days after fertilization) were examined. Part of the material was kindly lend by other institutes, another part was prepared by the author

Table 1. Data concerning the embryos studied

Number	Age of embryos in days	Length of embryos in mm*	Fixation	Direction of section	Thickness in microns	Remarks
T 52799	—	8	Formalin	Transverse	5	Tubal pregnancy
T 97551	—	8	Formalin	Transverse	14	Tubal pregnancy
E 1	—	10	Formalin	Transverse	10	Spontaneous abortion
BPA 108	42	11	Bouin-d'Hollande	Transverse	7	Curettage
S 39	—	11	Formalin	Transverse	10	Tubal pregnancy
ME	—	12	Formalin	Coronal	10	
S 4	—	12	Formalin	Transverse	10	
T 32530	—	13	Formalin	Sagittal	14	Ectopic pregnancy
S 10	—	13	Formalin	Transverse	10	Spontaneous abortion
BPA 113	44	13	Formalin (Holt)	Transverse	7	Curettage
E 153	—	14	Formalin	Transverse	10	Spontaneous abortion
S 62	—	14	Formalin	Transverse	14	Spontaneous abortion
T 81793	—	14	Formalin	Transverse	14	Ectopic pregnancy
BPA 112	46	14	Formalin (Holt)	Transverse	7	Curettage
S 8	—	15	Formalin	Transv./sag.	10	Spontaneous abortion
T 127776	—	15	Formalin	Sagittal	14	Ectopic pregnancy
E 65	—	16	Formalin	Transverse	10	Curettage
E 162	—	17	Formalin	Transverse	10	Ectopic pregnancy
BPA 101	47	17	Bouin-d'Hollande	Transverse	7	Curettage
S 9	—	17	Formalin	Transv./sag.	10	Spontaneous abortion
T 132870	—	18	Formalin	Sagittal	14	Ectopic pregnancy
BPA 107	51	19	Bouin-d'Hollande	Transverse	7	Curettage
T 86663	—	20	Formalin	Transverse	14	Curettage
S 47	—	20	Formalin	Transverse	10	Spontaneous abortion
S 51	—	21	Formalin	Transverse	10	Spontaneous abortion
E 13	—	22	Bouin	Transverse	10	Ectopic pregnancy
S 83	—	22	Formalin	Transverse	10	Spontaneous abortion
BPA 104	56	22	Formalin	Transverse	7	Curettage
BPA 109	56	22	Bouin-d'Hollande	Transverse	7	Curettage
BPA 100	54	22	Bouin-d'Hollande	Transverse	7	Curettage
S 50	—	23	Formalin	Transverse	10	Spontaneous abortion
BPA 111a	56	23	Bouin-d'Hollande	Transverse	7	Curettage; one of gemelli
BPA 111b	56	23	Bouin-d'Hollande	Transverse	7	Curettage; other of gemelli
E 139	—	24	Formalin	Transverse	10	Spontaneous abortion
BPA 102	60	—	Bouin-d'Hollande	Sagittal	7	Curettage
E 41	—	25	Carnoy	Transverse	10–20	
PL 4198	—	26	Formalin	Transverse	10	Spontaneous abortion
BPA 106	63	—	Bouin-d'Hollande	Transverse	7	Curettage
BPA 30	—	31	Formalin	Transverse	10	Spontaneous abortion
1472	—	33	Formalin	Transverse	7	Spontaneous abortion

* Embryos more than 15 mm in length were measured by their C–R length.

himself (Table 1)[1]. These embryos were fixed in Bouin's fixative (modified after D'Hollande) or in a 10 per cent formaldehyde solution neutralized by $CaCl_2$, buffered by phosphate and corrected as to its osmolarity by sucrose. The material was properly progressed, embedded in paraplast and serially sectioned at 7 microns. All embryos, except one, were cut transversely, because the axial structures were the most helpful landmarks for finding and following the often inconspicuous and variable lymphatics. Because no specific staining for the early lymphatic endothelium was available, a routine general tissue staining, namely Ehrlich's haematoxylin and azophloxin 0.05 per cent was employed.

In order to obtain reliable results from this rather simple method, it was of the utmost importance that series of sections were available of which the completeness was beyond any doubt. For, whereas in the elder embryos the lymphatics in most cases were easy to identify because of their appearance as large-meshed networks of irregular and wide thin-walled vessels, which suddenly may narrow considerably and never contain many blood cells, great problems were met in respect of the lymphatics in younger embryos. In these embryos some of the above mentioned characteristics are often missing in varying grades, and even if they do show up, they may be much less marked than in the elder ones. Besides, they may even differ in one and the same embryo and in different embryos of the same age. Fortunately all lymphatics have, besides these irreliable features, one characteristic in common, which can always be found, namely their almost complete isolation from the blood vessels. This is due to the fact that, although both structures are often lying very close together, not more than one or two very small and split-like connections between the two could be found. Before inferring to the occurrence of two seperate or almost separate vascular structures certainty about the completeness of the series had to be established beyond doubt. It was for this reason that the own material was prepared by the author himself.

For demonstrational purpose the main blood and lymph vessels of ten selected embryos were reconstructed by a method that is essentially the same as Gruenwald (1938) used in his study of the development of the inferior caval vein.

III. Observations

In the embryos collected and prepared as described, it was possible to study the development of the lymphatic system from the very beginning, when only a few inconspicuous small vessels could be found, up to the phase when all main lymph truncs known from the adult were completed.

Although in the development of the lymphatic system real distinct phases are lacking, it seemed desirable to make a division into 5 phases in order to obtain an appropriate description.

A. First Phase (Embryos from 8 to 12 mm Total Length)

Whereas in the youngest embryos of 8 mm on lymph vessels could be identified, in somewhat older embryos vascular structures were found, whose lymphatic character at one time was rather questionable and at another time was unmistakebly true.

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1 The T-numbered embryos belong to the collections of the Laboratory of Pathology, Department of Neuropathology (Head: V. D. W. Schenk, M.D.) and the Department of Anatomy (Head: Prof. J. Moll, M.D.) of the Erasumus University of Rotterdam; the E-numbered embryos are part of the collection of the Department of Anatomy and Embryology of the University of Utrecht (Head: Prof. W. J. van Doorenmaalen, M.D.); and the S-numbered embryos were lend from the collection of the Department of Anatomy and Embryology of the University of Leiden (Heads: Prof. J. Dankmeier M.D. (†) and Prof. J. M. F. Landsmeer).

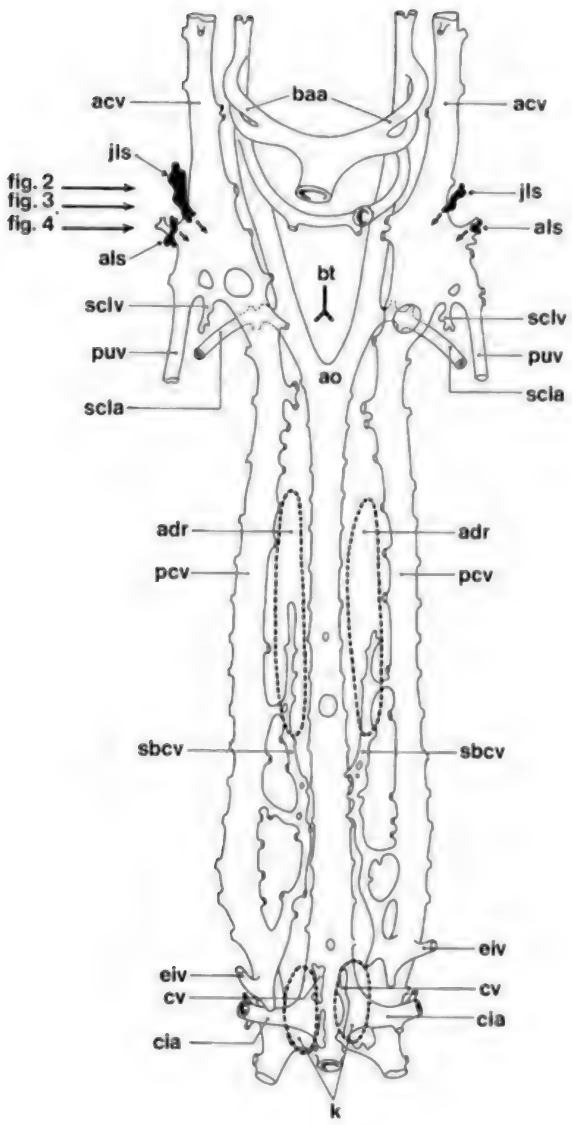

Fig. 1. × 35. Graphic reconstruction of the large prevertebral blood vessels and first lymphatic primordia of an 11 mm embryo, anterior view. The early jugular (*jls*) and axillary (*als*) lymph sacs are rather inconspicuous and blind-ending vessels communicating with the anterior cardinal veins (*acv*) via small and split-like apertures (↓)

The first lymphatics were observed in the rather extensive jugular region, where a short while before the large blood vessels had developed (Fig. 1). Here, the arterial system consists of the bilaterally symmetric branchial arteries bending dorsally to the dorsal aortae. From these aortae the dorsal intersegmental arteries arise, of which those originating just cranial to where both aortae fuse, are about to form the small subclavian arteries. Just lateral to the aortae the wide anterior cardinal veins are situated. These continue cranially into the head veins, receiving all blood from the rest of the head and from the neck region by several, mainly small, venules. These venules may be divided into the segmentally arranged dorsal branches, medial branches from the oesophageal, tracheal and bronchial structures and the vagus nerves, a few ventral branches

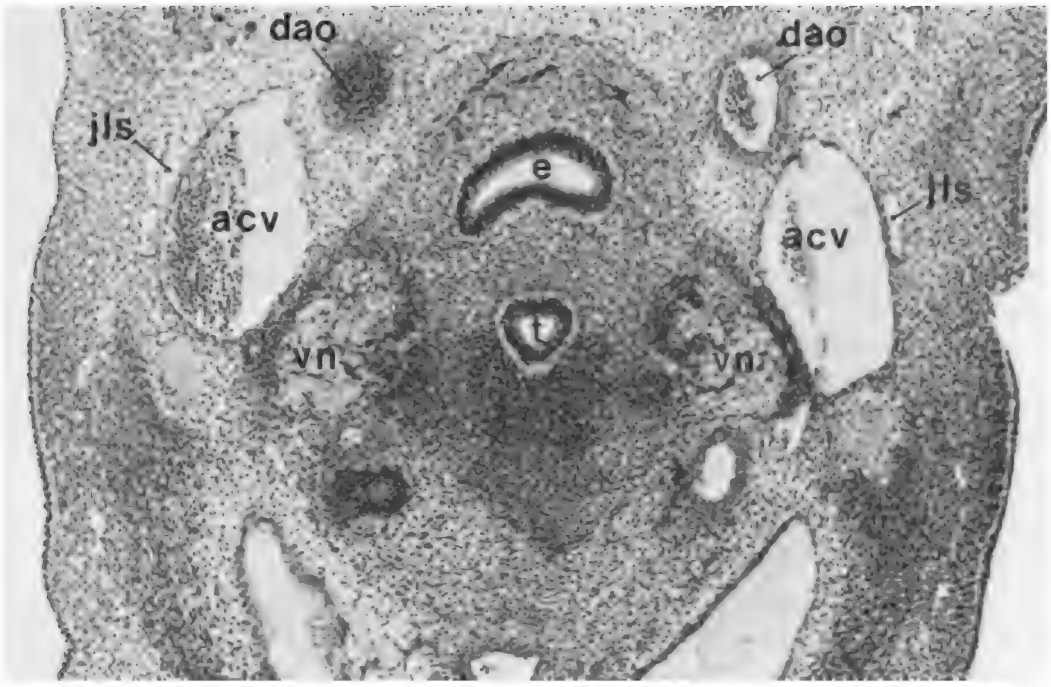

Fig. 2. × 60. Transverse section through the jugular region of an 11 mm embryo, at the level indicated in its reconstruction, Fig. 1. It shows the rather inconspicuous nature of the early jugular lymph sacs (*jls*)

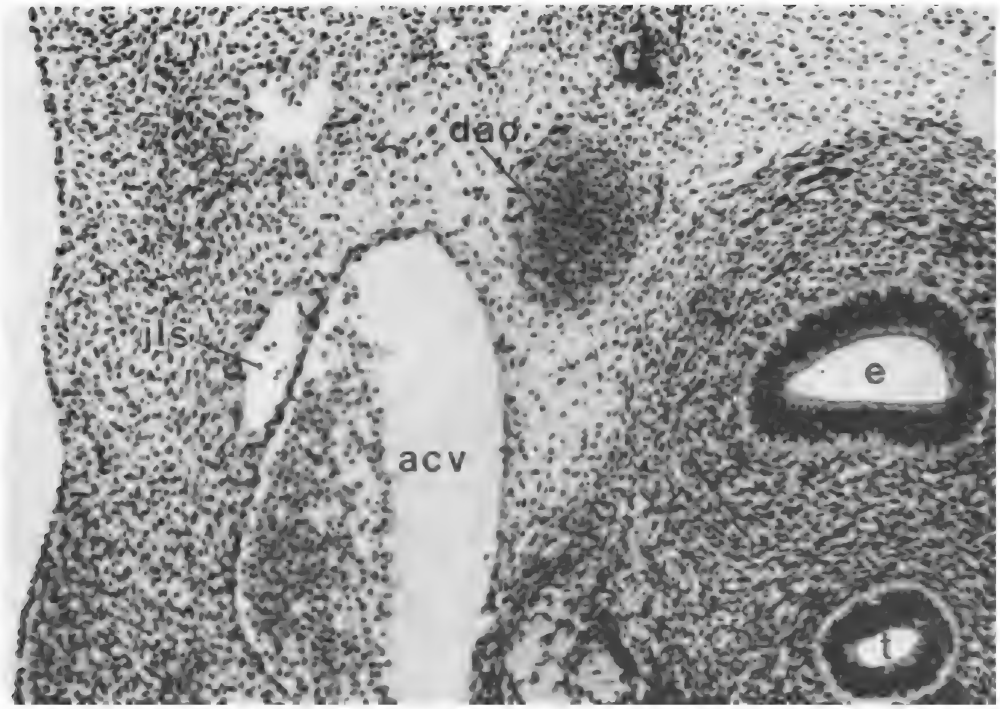

Fig. 3. × 100. Detail of the right jugular lymph sac (*jls*) shown in Fig. 2. It shows the close position of the jugular lymph sac to the wall of the anterior cardinal vein (*acv*)

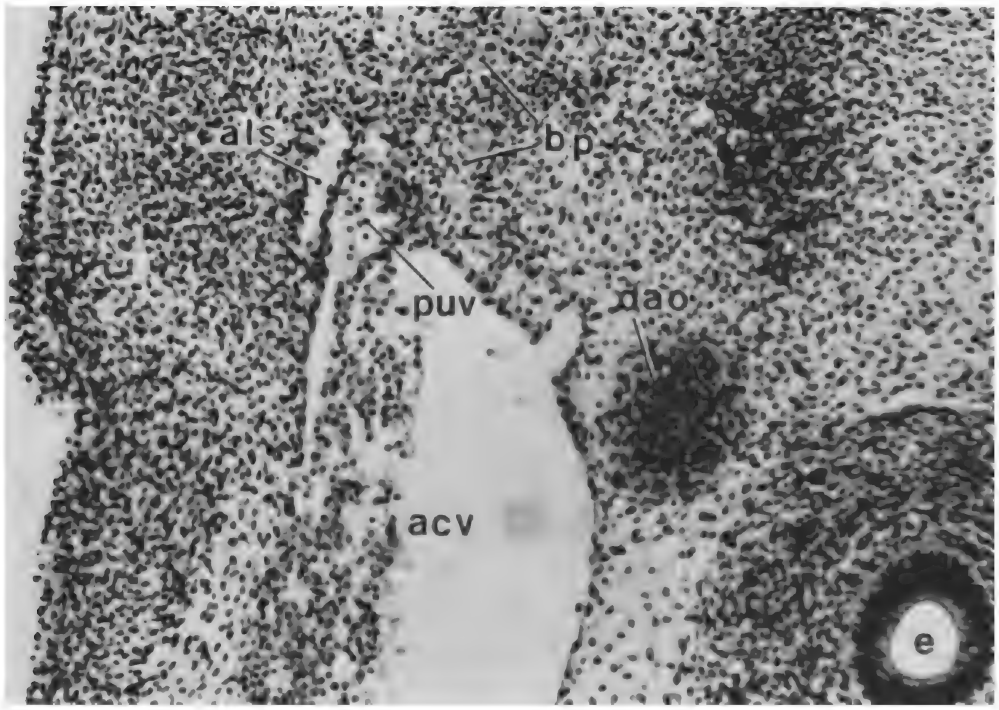

Fig. 4 $\times$ 100. Transverse section at a more caudal level through the same embryo as shown in the previous figures. An extension of the right axillary lymph sac (*als*) is situated just lateral of the primitive ulnar vein (*puv*)

from the facial region and the tongue and several lateral branches from the rapidly differentiating lateral musculature of the neck and the skin. The most caudal of these lateral branches, which is the largest of all, has its outlet in the most caudal part of the anterior cardinal vein just cranial to the confluence of the anterior and posterior cardinal veins. This "primitive ulnar vein" extends into the developing fore limb and the adjacent part of the lateral body wall. Passing between the cervical spinal nerves 4 and 5, it runs laterally to the brachial plexus of nerves. In addition to this channel, in embryos of 11 mm total length, medial to the brachial plexus, a second venous network is formed representing the beginning of the subclavian vein.

In these 11 mm embryos some very peculiar vascular structures were observed in the jugular region on both sides of the embryos. They were situated near the lateral branches cranial to the primitive ulnar vein and appear as irregular and wide vessels, which, notwithstanding their close proximity, are completely separated from the anterior cardinal veins except for one narrow cleft-like aperture. These vessels differ clearly from other, venous, tributaries. Because of their form and their topographical relationship as compared with older embryos, these vessels were considered to represent very early lymphatics, which were named "*jugular lymph sacs*" (Figs. 2, 3).

In the same embryo, however, yet another bilaterally arising lymphatic primordium was observed. It is situated somewhat more caudally in the cranial angle of the confluence of the primitive ulnar vein and the anterior cardinal vein (Fig. 4). In one of these 11 mm embryos it lies amid a small venous network and is easily mistaken for a very complex venous tributary. After careful examination,

however, it proved to be built up out of two different plexuses. One of these was considered to be venous, mainly because of its wide communications with the anterior cardinal vein, the other, which was almost isolated from the veins was thought to be lymphatic in character. This supposition proved to be in accordance with a situation met in the other 11 mm embryo, which is somewhat more developed and showed a real lymph sac having the same position. Because of its later extension into the axillary region, it was named "*axillary lymph sac*".

In none of the embryos of this category other lymphatic primordia seemed to exsist.

B. Second Phase (Embryos from 12 to 15 mm Total Length)

From now on the jugular and axillary lymph sacs enlarge rapidly (Fig. 5). The *jugular lymph sacs* soon extend cranially almost to the mesenchymal condensations, which represent the early base of the skull. Outgrowth into a medial and lateral direction is still limited, except for the most caudal part of the sac, where sprouts are formed, which somewhat later communicate with cranial extensions of the axillary lymph sac. The jugular lymph sac lies very close to the anterior cardinal vein, which name after the forming of the external jugular vein, is changed partly into the "internal jugular vein" and partly into the "superior caval vein". As before, only one cleft-like communication with this vein was found, which because of the development of the external jugular vein has to be sought in the angle between this vein and the internal jugular vein (Fig. 6). The opening is guarded by small valves, which are in fact the walls of both confluent veins. In some embryos, besides this rather wide opening, other, small, connections appeared to exist somewhat more cranially or medially.

As mentioned above, the *axillary lymph sac* grows out especially in a lateral direction. At first it parallels the primitive ulnar vein, thus reaching the axillary region, where this vein soon communicates with the rapidly enlarging subclavian vein (Fig. 6A). From now on the primitive ulnar vein decreases in size at an increasing rate, until the whole of the blood of the fore limb is drained by the subclavian vein. That part of the axillary lymph sac, which before paralleled the primitive ulnar vein, however, continues to exist, retaining its former position lateral to the brachial plexus (Fig. 6B). From the cranial part of the axillary lymph sac other, at first small, but later wide prolongations extend cranially, lateral to the internal jugular vein. They are seperated from this vein by the proximal part of the external jugular vein, a branch of the thyrocervical artery and the most caudal part of the jugular lymph sac together with its venous communication (Fig. 1).

The axillary lymph sac itself opens into the vein more caudally: at first near the confluence of the primitive ulnar vein and the internal jugular vein, and later, after the disappearance of the former, in the angle between the subclavian vein and the internal jugular vein (Fig. 8).

In embryos as short as 13 mm total length, at one or both sides of the embryo around the proximal part of the external jugular vein, anastomoses have been formed between cranial extensions of the axillary lymph sac and lateral branches of the caudal part of the jugular lymph sac. These connections widen up and after a short period it is impossible to distinguish the former two primordia.

Only the occurence of two lymphatico-venous anastomoses reminds of the derivation of this "*jugulo-axillary lymph sac*" (Figs. 5 and 6B).

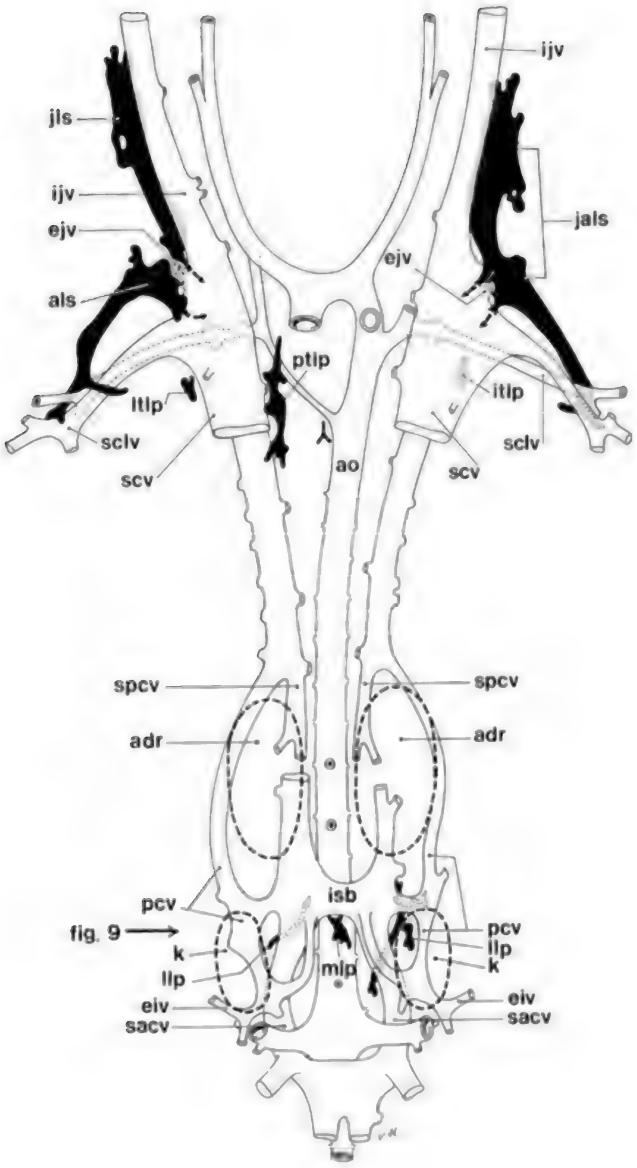

Fig. 5. × 30. Graphic reconstruction of the lymphatic primorida and the main prevertebral blood vessels of a 13 mm embryo, anterior view. Development has progressed rapidly as compared with Fig. 1. On the left side the large and easily identifiable jugular (*jls*) and axillary (*als*) lymph sacs have fused, forming one jugulo-axillary lymph sac (*jals*). Still other primordia have developed: from cranial to caudal: the internal thoracic (*itlp*), paratracheal (*ptlp*), mesenteric (*mlp*) and lumbar lymph plexuses (*llp*)

In most of the embryos of this category, other lymphatic primordia were observed. Bilaterally, near the confluence of the early developing internal thoracic veins and the superior caval vein, small and inconspicuous spindle-shaped vessels have appeared (Fig. 5). Connections with the veins could not be identified with certainty. In their lumina varying numbers of blood cells are present, the grade of filling being independent from that of the neighbouring veins. Because of their topographical relationship with and their later extension along the internal thoracic artery and veins, these lymphatic primordia were named "*internal thoracic lymph plexuses*".

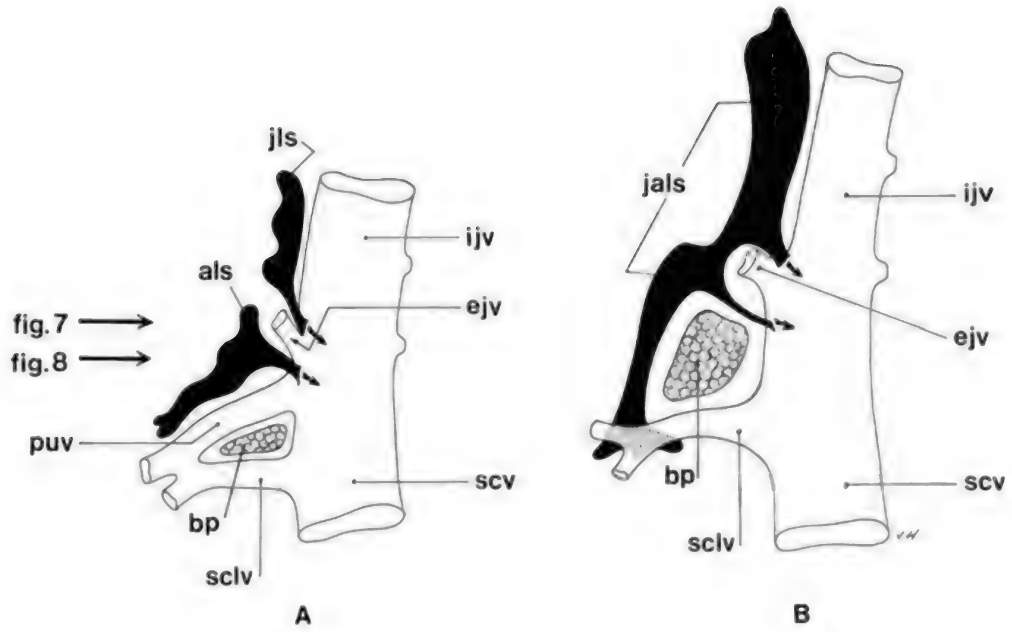

Fig. 6A and B. Schematic drawings of the jugular (*jls*) and axillary (*als*) ly mph sacs o embryos of 12 mm (A) and 14 mm (B) demonstrating the changes, which occur after the fusion of both primordia and after the regression of the primitive ulnar vein (*puv*). Anterior view

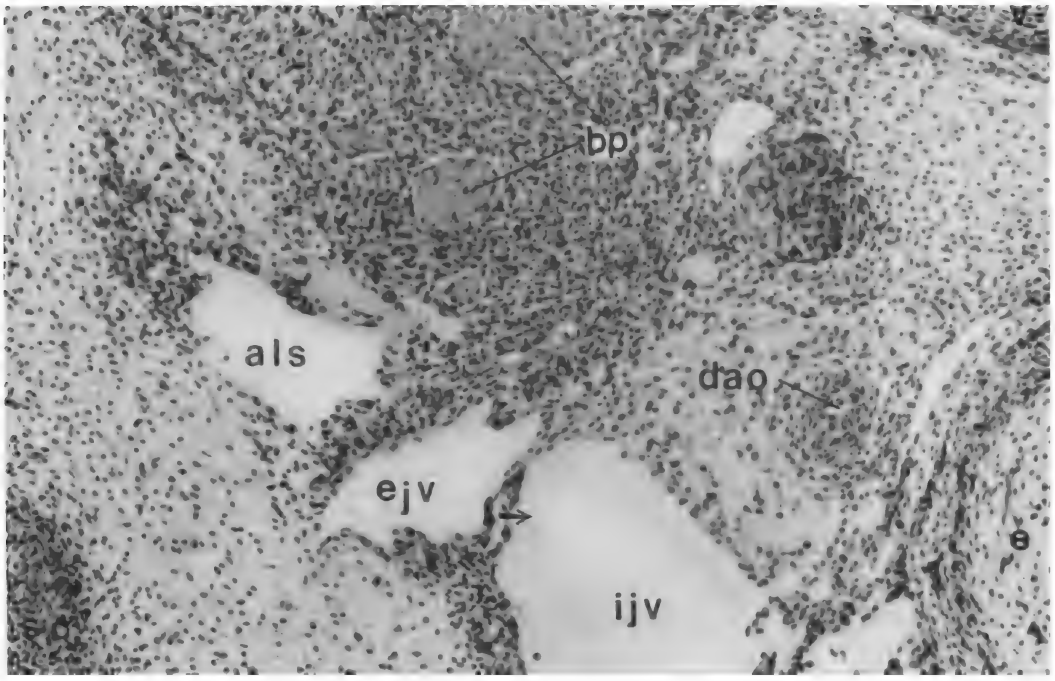

Fig. 7. × 160. Transverse section of a 13 mm embryo showing the lymphatico-venous communication (↓) of the right jugular lymph sac in the upper angle between the external (*ejv*) and internal jugular veins (*ijv*). The cranial part of the axillary lymph sac (*als*) is lying just lateral to the external jugular vein. Level indicated in Fig. 6

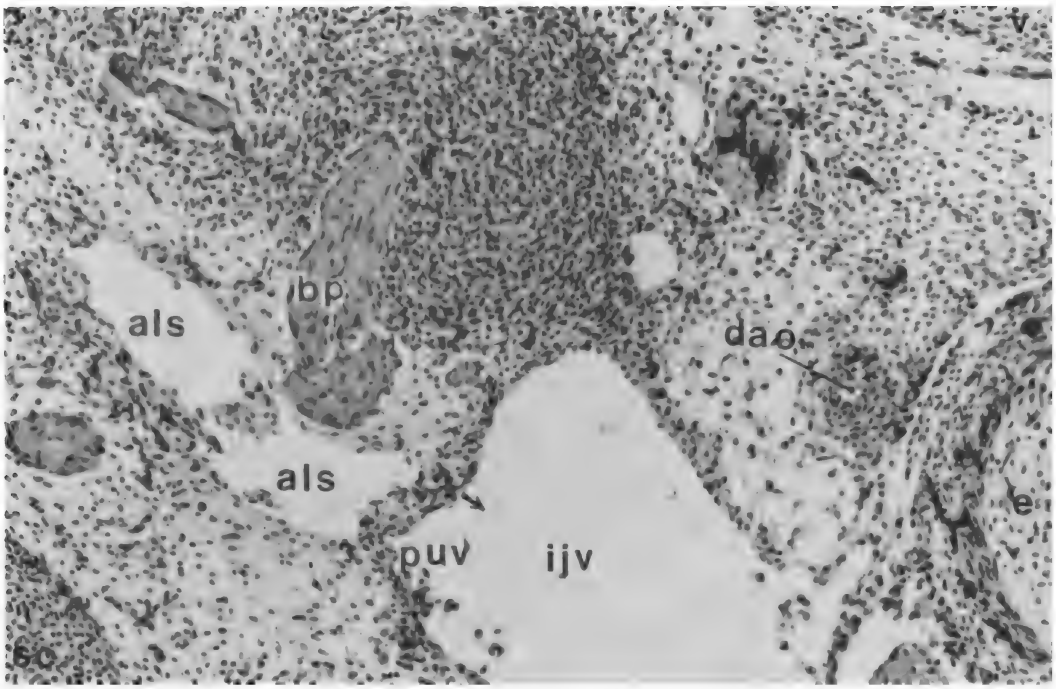

Fig. 8. × 160. Transverse section at a more caudal level of the same embryo as shown n Fig. 7. It demonstrates the small lymphatico-venous communication (↓) of the right axillary lymph sac (*als*) in the upper angle between the primitive ulnar vein (*puv*) and the internal jugular vein (*ijv*). Level indicated in Fig. 6

At the same time and the same level, but on the other, medial, side of both superior caval veins, yet another pair of primordia have arisen (Fig. 5). In nearly all of the embryos, the one on the right side is easy to discern, whereas in most cases the left primordium could hardly be identified, probably because of its small calibre. This bilateral primordium is situated near the outlet of the most caudal of the medial branches of the superior caval veins, which are the tracheobronchial veins. Form and position of these primordia change as a result of the caudalward movement of the heart and hili of the lungs, which is accompanied by lengthening of the superior caval veins and the trachea. Thus, these nearby lymphatics also become more protracted. They become irregular and plexiform structures situated on both sides between the superior caval vein, the vagus nerve, the trachea and most ventrally the pericardial cavity, extending from the level of the ductus arteriosus to the level of the bifurcation of the trachea. In this phase but a few small branches reaching the pulmonary arteries are observed. Connections with the veins could not be ascertained, but sometimes pictures highly suggestive for the existence of small, perhaps obliterated, openings were met. Their contents vary from no blood cells to large amounts of blood. Because of their position near the trachea, they were named "*paratracheal lymph plexuses*".

During this phase an important transformation takes place in the abdominal region (Fig. 5). Here, the posterior cardinal veins are "pushed" aside by the growth of the adrenal glands and by the enlargement and change in position of the kidneys into a cranial and latero-dorsal direction. These abdominal portions of the posterior cardinal veins are replaced by new channels formed medial to these

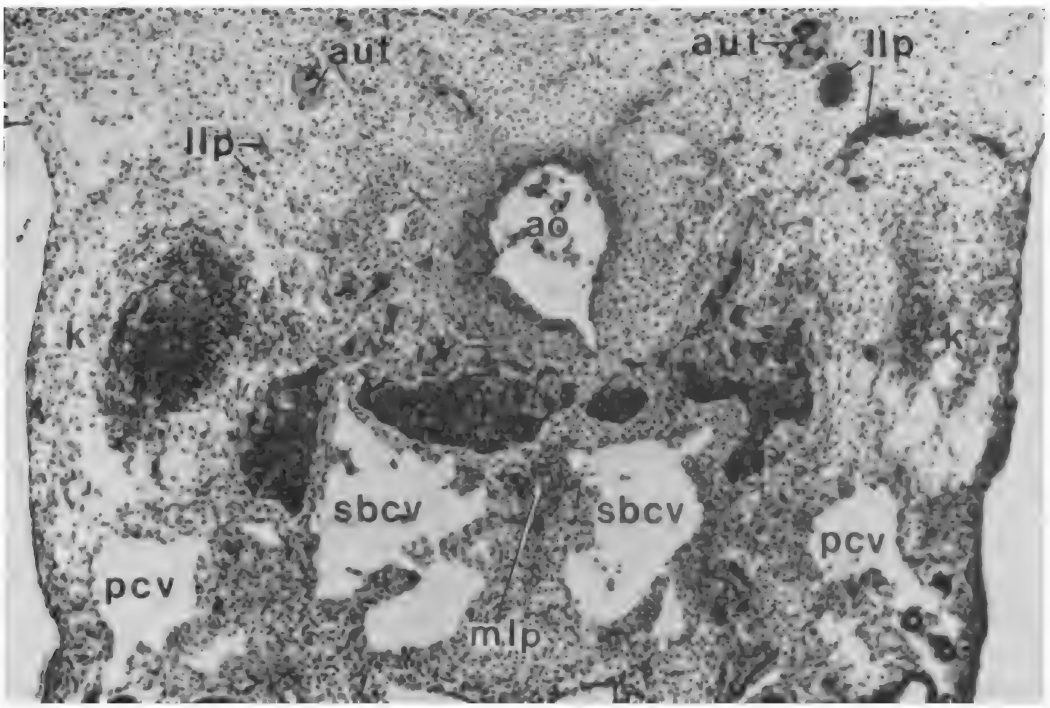

Fig. 9. × 100. Transverse section through the abdomen of a 13 mm embryo at the level indicated in the reconstruction, shown in Fig. 5. It shows an early mesenteric lymph plexus (*mlp*) situated between two wide subcardinal veins (*sbcv*). On both sides early lumbar lymph plexus (*llp*) are lying dorso-medial to the upper pole of both kidneys (k) and dorso-lateral to the abdominal aorta (ao)

organs. Thus, dorso-medial to the adrenal glands, the supracardinal veins develop as a downward extension of the cranial "thoracic" part of the posterior cardinal veins receiving the blood from the lower intercostal veins. Almost in the same way another pair of longitudinal channels is formed dorso-medial to the kidneys as an upward extension of the cranial part of the sacrocardinal veins, which previously connected the previous ischiadic and caudal veins with the posterior cardinal veins. These extensions collect the blood form the first lumbar veins, which previously was brought directly into the posterior cardinal veins by the dorsal segmental veins. Besides these channels situated dorsolateral to the aorta, yet another system of symmetric veins has developed, namely the subcardinal veins. Shortly after their development, a communication is established between the right subcardinal vein and the hepatic sinusoids. Later, after the development of the intersubcardinal anastomosis and the establishment of large communications between each of these veins seperately and the sacrocardinal veins of the same side, an alternative pathway is formed from the legs and the pelvic and abdominal regions to the heart.

Amid the meshes of the, initially plexiform, intersubcardinal anatomosis, which is situated in front of the abdominal aorta between the stems of the coeliac and superior mesenteric arteries, the next lymphatic primordium had appeared (Figs. 5 and 9). It was found in every embryo of this group: in the younger as very discrete and somewhat spindle-shaped vascular structures, in the older embryos as small, but true, lymph plexuses. In most embryos they are still

confined to the proximity of the intersubcardinal anastomosis. In those embryos in which sprouts have started to develop, small prolongations reach the proximal parts of the coeliac and superior mesenteric arteries. No definite lymphatico-venous anastomoses could be identified. During this phase this primordium is mostly filled with blood cells. Because of its position near and its subsequent outgrowth into the mesentery, it was named "*mesenteric lymph plexus*".

Many more difficulties were met in identifying another paired lymphatic primordium. It develops at the same time and in the same region as the above mentioned new venous channels dorso-lateral to the abdominal aorta. The first indication of these lymphatic primordia is observed on both sides near the splanchnic nerves at the level of or immediately above the confluence of the sacrocardinal veins and the subcardino-sacrocardinal anastomoses. As mentioned before, it is in this region that new connections are established between these veins and the first lumbar veins.

In the younger embryos some very small and isolated vascular structures were sometimes observed, where in the older embryos slender and more plexiform structures exist (Figs. 5 and 9). In most cases these vessels differ from neighbouring veins in that they are almost completely filled with blood cells. In those embryos, in which this difference is very distinct, it was possible to trace them out and to confirm their isolation from the venous system. After their position near the lumbar vessels these primordia were named "*lumbar lymph plexuses*".

C. Third Phase (Embryos from 15 to 20 mm C-R Length)

In the neck region, both *jugulo-axillary lymph sacs* are expanding rapidly (Fig. 11). In each of them one may now discern these portions: a jugular portion just lateral to the internal jugular vein, a second, axillary, portion in the axillary region near the axillary vessels, and finally a relatively slender connection between both sac-like parts. As stated before, both large portions do not correspond with the former jugular, respectively axillary lymph sacs, because the most medial part of the axillary lymph sac is now incorporated in the jugular part. Except for the slender and blind-ending process, which reaches to the jugulo-subclavian confluence, having in its tip the original venous communication of the axillary lymph sac, it is from now on impossible to separate both original parts. On the other hand, one may say without restriction that the connection between the lower larger portions of the sac, which has held its former position, is wholly derived from the axillary lymph sac.

From the jugular portion of the jugulo-axillary lymph sac large extensions develop mainly into a cranial and dorso-lateral direction. Growing out between the sternocleidomastoid and trapezius muscles they have reached the subcutaneous area of the posterior triangle of the neck. Other sprouts extend inbetween the cervical spinal nerves 2, 3 and 4, enclosing them by enlarging and fusing, until at last the nerves seem to pass through foramina in one, large, lymph sac. Still other, medial, sprouts extend into the loose connective tissue, which surrounds the oesophagus. In the jugular portion of the jugulo-axillary lymph sac, both previously observed lymphatico-venous communications were found: the one formerly between the jugular lymph sac and the vein at the confluence of the external and internal jugular veins, and the other between the former axillary

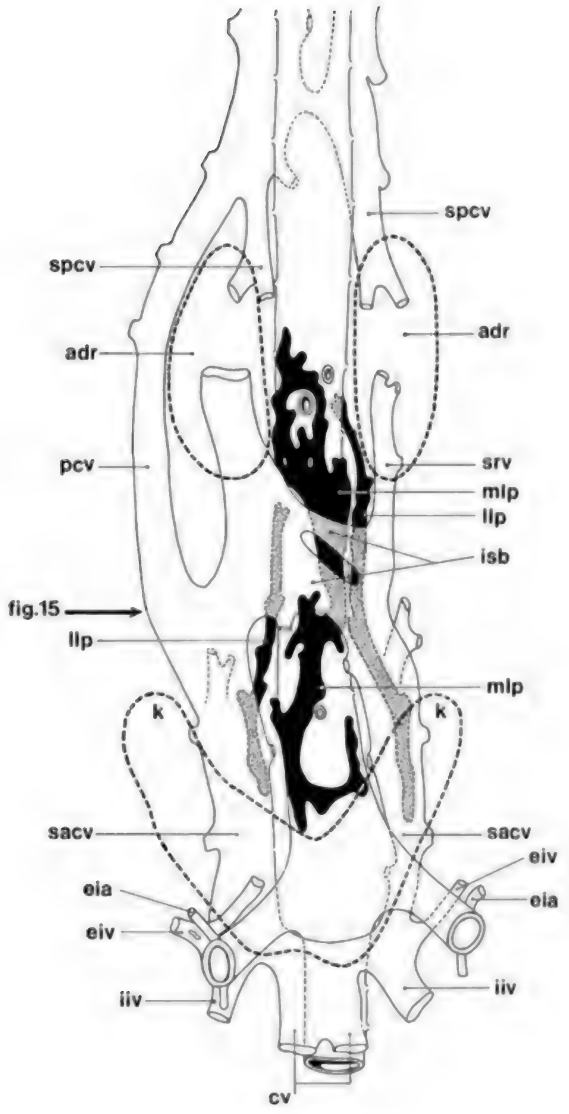

Fig. 10. × 70. Graphic reconstruction of the abdominal lymphatic primordia and main prevertebral blood vessels of a 16 mm embryo, anterior view. It shows a nice transitional phase of the venous system from a postcardinal to an inferior caval system of veins. The mesenteric lymph plexus (*mlp*) is rather large as compared with the small and slender lumbar lymph plexuses (*llp*)

lymph sac and the vein at the confluence of the subclavian and internal jugular vein. Whereas the first shares the general enlargement of the lymph sac, the other stays behind in growth and may even be overlooked sometimes.

The axillary portion enlarges too, although to a lesser degree. It comes to its greatest extension ventral and medio-ventral to the axillary artery and vein, and just outside the first ribs. Small sprouts grow out along the proximal parts of all arterial and venous branches in this region.

In all embryos the jugulo-axillary lymph sac is almost empty.

In three embryos of this category the *internal thoracic lymph plexuses* have not been observed yet. In other embryos, however, rather wide vascular structures were met, which were unmistakebly lymphatic of character. They are situated as before

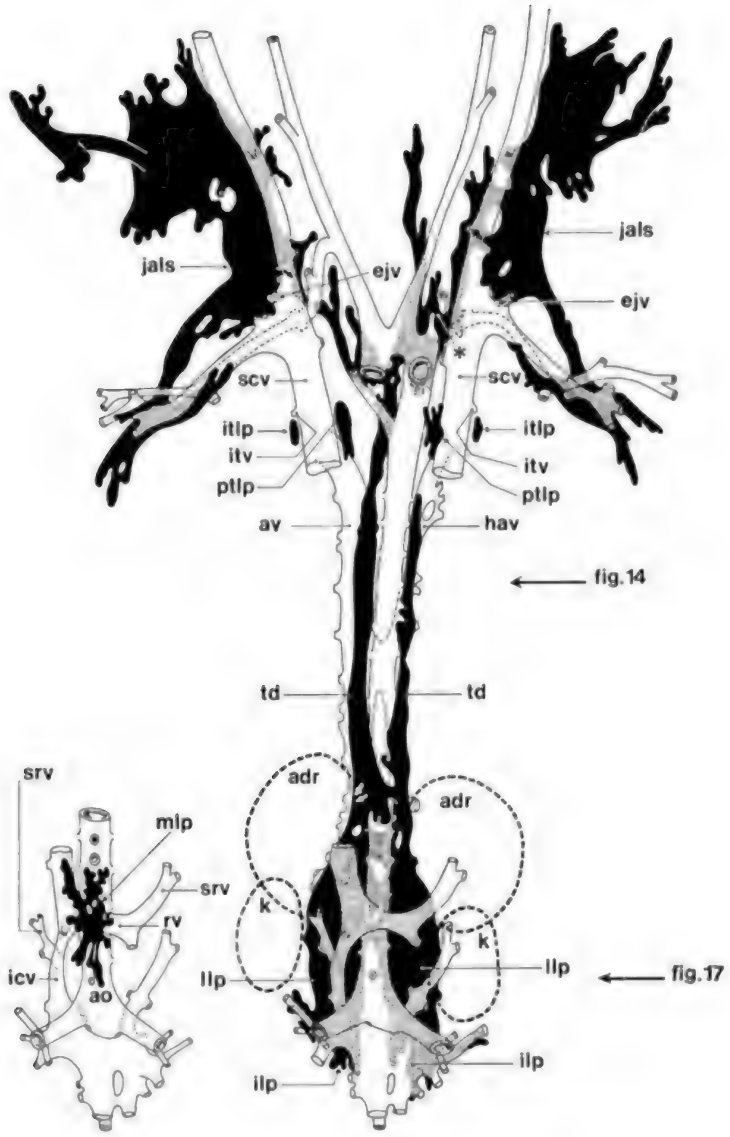

Fig. 11. × 20. Graphic reconstruction of the large prevertebral blood and lymph vessels of a 19 mm embryo, anterior view. It demonstrates well the unusual large and far-developed jugulo-axillary lymph sacs (*jals*), thoracic ducts (*td*), lumbar (*llp*) and iliac lymph plexuses (*ilp*), which already communicate. Whereas the iliac lymph plexuses are almost incorporated in the enormous lumbar lymph plexuses and wide connections were formed between these primordia and the thoracic ducts, but one very small communication is found between a cranial extension of the right thoracic duct and a caudal extension of the left jugulo-axillary lymph sac (indicated by asterisk). Another cranial extension of the duct ends at a small distance from a caudal extension of the right jugulo-axillary lymph sac. At the level of the diaphragm between the right thoracic duct and the azygos vein (av) lymphatico-venous communications (white ↓ near adr) were detected. The size of the internal thoracic (*itlp*), paratracheal (*ptlp*), subtracheal (not shown) and mesenteric lymph plexuses (*mlp*) is in accordance with similar primordia in other embryos of nearly the same age and length

against the ventro- and dorso-lateral sides of the superior caval veins near the openings of the internal thoracic veins (Fig. 11). These lymphatics are almost empty and connections with the veins were not observed.

In all embryos of this stage a paired *paratracheal lymph plexus* could be demonstrated. In some cases such a primordium looks as if it originated from two separate primordia, which had recently been united. In comparison with the plexuses of the previous phase, form and position are essentially the same (Fig. 12). As before, they share the nearby structures in growth and some small branches have developed around the pulmonary arteries (Fig. 11). Sometimes the plexuses are empty, sometimes they are partially filled with blood. No connections seemed to exist with the veins. Nor did connections seem to exist between these paratracheal lymph plexuses and another nearby lymph plexus, which in some embryos was found ventral to the caudal half of the oesophagus and just caudal to the bifurcation of the trachea. Although the presence of small anastomoses between both plexuses could not be excluded with certainty (because of difficulties involved in examining the densely structured connective tissue around the bronchi), the whole picture is very suggestive of another isolated primordium. It was named "*subtracheal lymph plexus*". It contains but very small numbers of blood cells.

In all embryos of this group new lymphatic primordia were also observed near the thoracic aorta. Already during the previous stage some vascular structures in this region had been noted, which were rather conspicuous because of differences in form and contents as compared with nearby veins. At that moment, however, no sufficient proof was available to establish their lymphatic character. In fact, the same is true for some similar vessels in the younger embryos of this category. But in the older embryos distinct vessels were found, which were clearly lymphatic in nature. At first these are still small and slender lymphatics, isolated from the veins and often uncommonly distended by blood cells, which is often in sharp contrast with almost empty neighbouring veins (Fig. 13). They are situated close to the ventral side of the azygos and hemiazygos veins at or somewhat caudal to the level, where both the first transverse anastomoses between these veins develop, and the supracardinal system had started to grow caudalward. Sometimes besides these primordia yet other, small, but "suspect" structures were observed more cranially along the thoracic aorta. In some cases it is almost certain that one deals with separate lymphatic primordia.

These primordia originating and developing in the region of the future thoracic duct, later become more protracted in form. In a rather far developed 19 mm embryo, bilaterally real thoracic ducts were observed, which anastomose cranially with the left jugulo-axillary lymph sac and caudally with both lumbar lymph plexuses (Figs. 11 and 14). Still more interesting is the observation that these lymphatics are completely filled with blood cells. It is most probably caused by two open communications between the caudal part of the right thoracic duct and the supracardinal portion of the azygos vein (Fig. 11).

In the abdominal region, where the paired lumbar lymph plexuses and the unpaired mesenteric lymph plexus can easily be identified now, yet another lymphatic primordium has appeared.

The rebuilding of the venous system, started in the previous period, has progressed steadily (Figs. 10 and 11). Concurrently with the increase in size of both adrenal glands and kidneys, the remnants of the posterior cardinal veins have disappeared almost completely. The subcardinal veins, sacrocardinal veins and the anastomoses between those veins have greatly enlarged with a marked preponderance of the vessels of the right side. The subcardino-sacrocardinal ana-

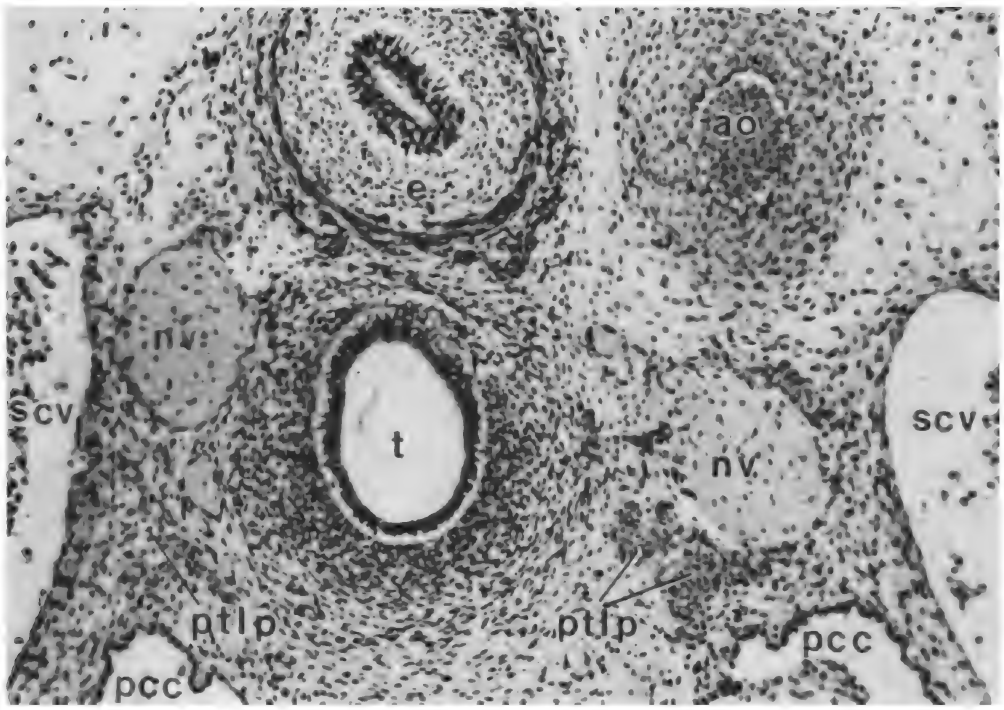

Fig. 12. × 100. Transverse section through the upper mediastinum of a 17 mm embryo to show the typical position and topographical relationships of both paratracheal lymph plexuses (*ptlp*) early in their development

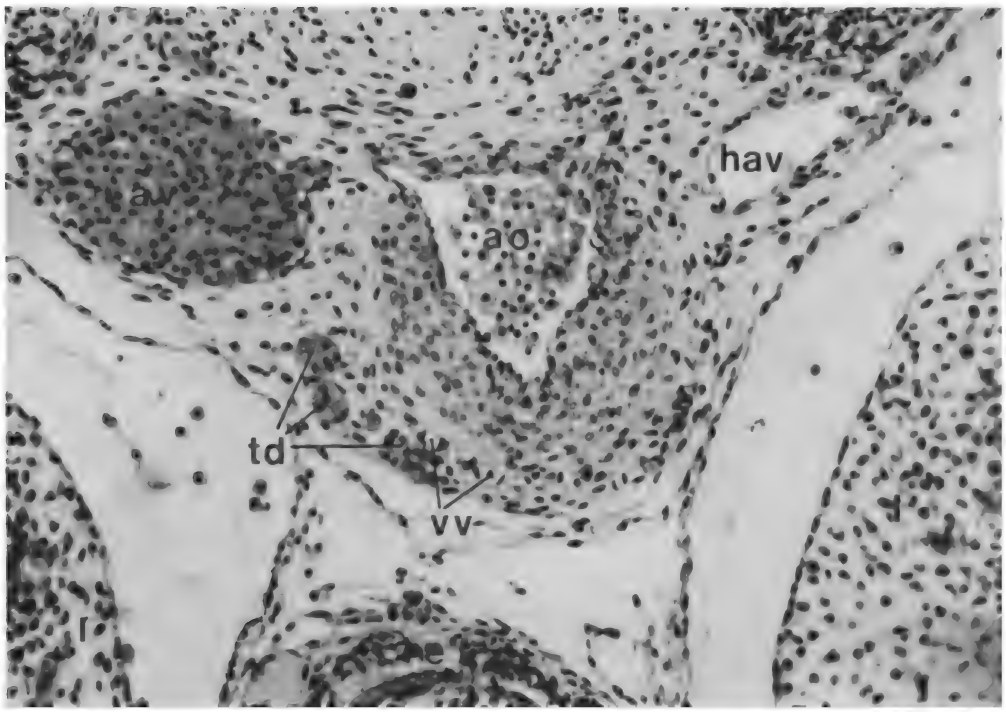

Fig. 13. × 250. Transverse section at a more caudal level of the same embryo as shown in Fig. 12. It shows small blood-packed vessels representing an early right thoracic duct (*td*). Note the contrast between these vessels and the empty periaortic venules (*vv*)

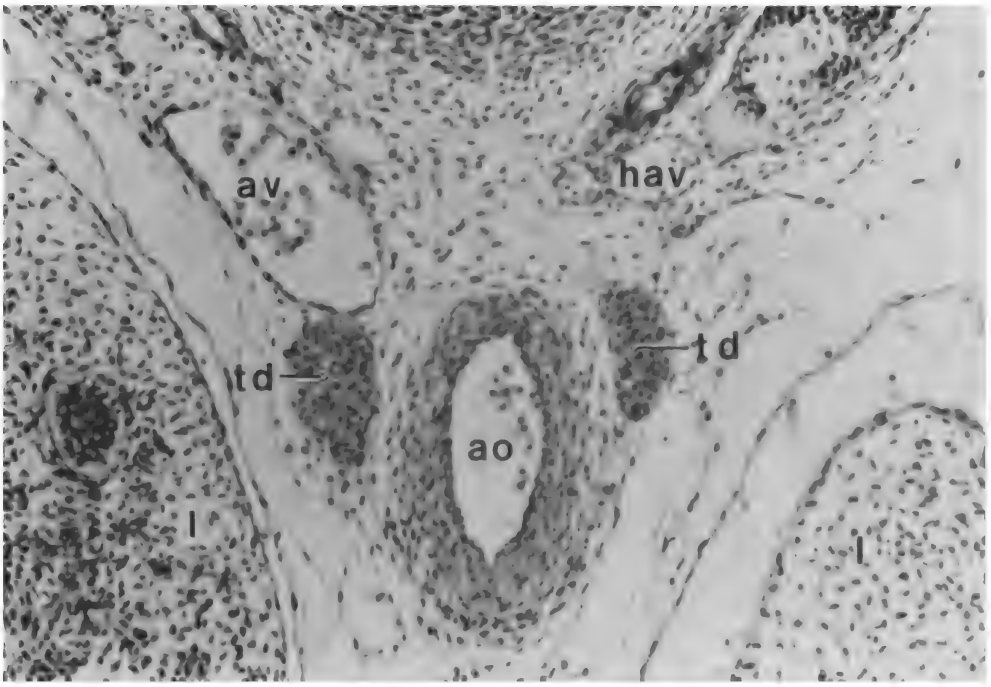

Fig. 14. × 250. Transverse section of a 19 mm embryo at a level indicated in the reconstruction, shown in Fig. 11, to show large thoracic ducts (td) filled with blood cells

stomoses take a more horizontal course, while at the same time the subcardinal veins lengthen. The external iliac veins, formerly feeding their contents into the posterior cardinal veins, have changed their outlet and now open into the common iliac veins, which have been formed by fusion of the caudal part of the sacrocardinal veins and the caudal veins.

As to the lymphatic primordia, in all embryos plexiform or even sac-like mesenteric lymphatics have been formed around the intersubcardinal anastomosis (Figs. 10, 11 and 15). This *mesenteric lymph plexus* is always widest near the anastomosis. From this portion, slight extensions have grown out along the subcardinal veins (which are named from now on: inferior caval vein and left suprarenal vein). In some embryos, these sprouts reach as far as the origin of the coeliac artery, the inferior mesenteric artery and, laterally, to the hilar regions of both mesonephroi, which still occupied a rather medial position. In this phase, no connections with other lymphatic primordia or with the veins were observed. In most embryos the plexus is completely filled with blood.

With but a few exceptions, in most embryos distinct *lumbar lymph plexuses* are observed dorso-lateral to the abdominal aorta and dorsal to the sacrocardinal veins and subcardino-sacrocardinal anastomoses (Figs. 10, 11, 15, 16 and 17). As before, they encircle the lumbar veins and splanchnic nerves. Except for two embryos, which show but small and inconspicuous vessels and one embryo (already mentioned before because of its far developed thoracic ducts), which demonstrates large labyrinth-like vascular structures (Fig. 17), all embryos have slender and rather simple plexuses extending from a little beneath the diaphragm to the level where the inferior caval vein splits into both common iliac veins. In some embryos they seem to extend even more caudally, but as will be demonstrated

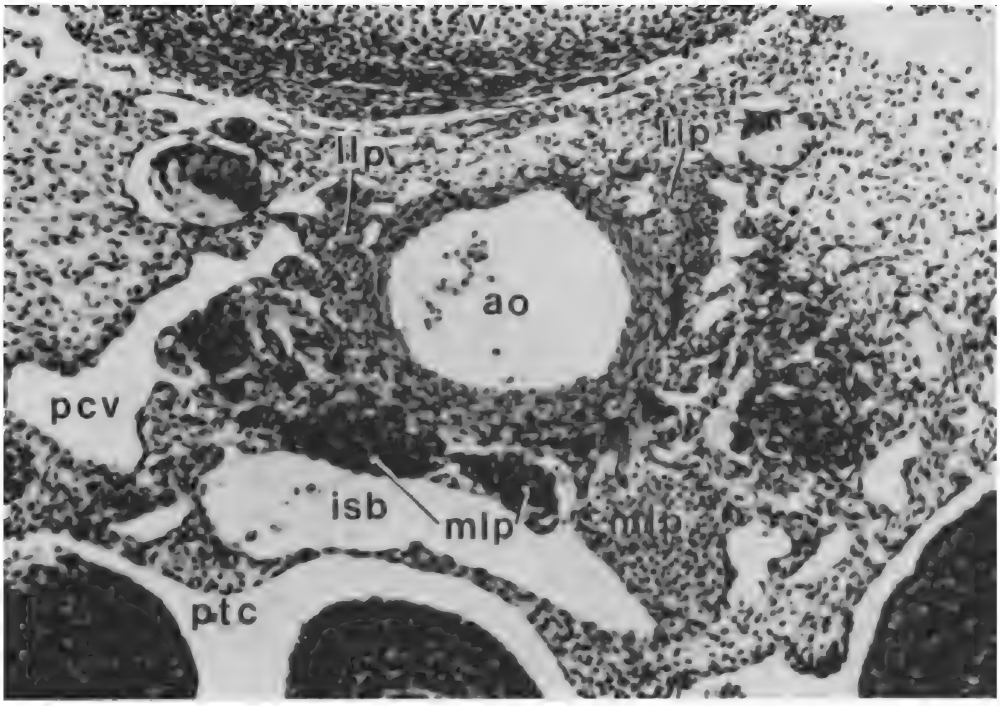

Fig. 15. $\times$ 100. Transverse section of a 16 mm embryo at the level indicated in the reconstruction, shown in Fig. 10. Note the contrast in contents of the blood-filled mesenteric (*mlp*) and lumbar lymph plexuses (*llp*) and of the blood vascular system from which the blood probably escaped when the embryo was removed

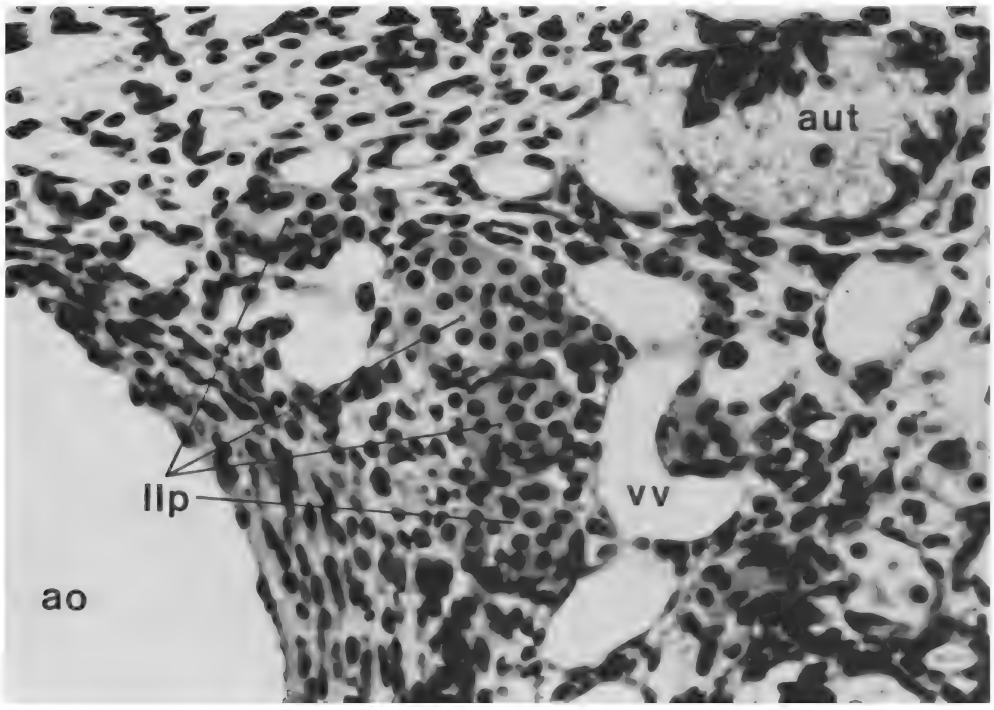

Fig. 16. $\times$ 250. Detail of Fig. 15 showing the very close position of the left lumbar lymph plexus (*llp*) to the periaortic venules (*vv*)

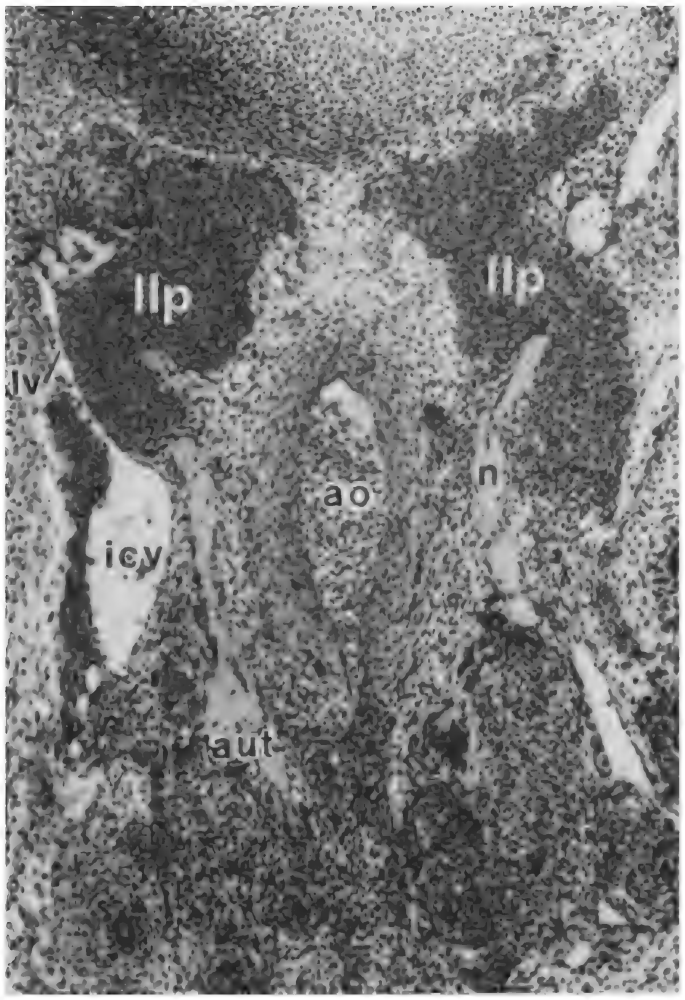

Fig. 17. × 100. Transverse section through the abdomen of a 19 mm embryo at the level indicated in the reconstruction, shown in Fig. 11, showing the enormous labyrinth-like lumbar lymph plexuses (*llp*) traversed by the lumbar veins (*lv*) and nerves (*n*)

later, these most caudal parts represent a separate bilateral lymphatic primordium, which afterwards has fused with the lumbar lymph plexuses (Fig. 11). No connections with other primordia were found to exist, except for the afore-mentioned 19 mm embryo, in which they are in continuity with the thoracic ducts (Fig. 11). In most embryos the lumbar lymph plexuses contain large numbers of blood cells (Figs. 15, 16 and 17). It was not possible, perhaps as a result of the complexity of both lymphatic and venous networks intermingled with each other, to determine the occurrence of lymphatico-venous communications.

The above mentioned most caudal lymphatic primordia proved to be situated ventral, ventro-lateral and lateral to the most caudal parts of the sacrocardinal veins, later inferior caval vein and proximal parts of the common iliac veins, near the outlet of the external iliac veins. In a minority of the embryos of this category, no primordium on either side could be identified. In the other embryos they vary in form from sac- or labyrinth-like vascular structures to slender and plexiform vessels. Small sprouts either parallel the proximal parts of the external

iliac veins or extend medially, crossing the common iliac veins. Because of their position near the iliac veins and arteries, these primordia were named "*iliac lymph plexuses*". In most embryos they are still isolated from other primordia. In some embryos however, anastomoses with the lumbar lymph plexuses were formed, either on one side or on both sides. In these iliac lymph plexuses variable amounts of blood were observed.

D. Fourth Phase (Embryos from 20 to 25 mm C-R Length)

During this phase the various lymphatic primordia enlarge, fuse and begin to send out sprouts into more peripheral regions (Figs. 18, 19 and 20). No indications were found that other new primordia were developing.

In the neck and axilla the jugular and axillary portions of the *jugulo-axillary lymph sacs* expand still further, whereas the connections between them lag behind and thus, in a relative sense, decrease in diameter.

The jugular portion fills nearly all of the region enclosed by the internal jugular vein, the longi and scaleni muscles, the sternocleidomastoideus and the trapezius muscles. It is traversed by the second, third and fourth cervical spinal nerves. A large extension passing between the scaleni muscle splits into an extensive large-meshed network in the posterior triangle of the neck (Fig. 21). Other lymphatics have started to grow out along most of the branches of the internal jugular vein, especially along the external and anterior jugular veins, and the linguofacial vein. The medial processes augment in number and extension growing out into the peri-oesophageal region. By the most caudal of these sprouts, which even in the younger embryos of this category often have a remarkable large extension, communications become established between the jugulo-axillary lymph sacs and other, more caudally situated, primordia (Figs. 18, 19 and 20). Thus, at the left side, frequently connections have been formed mostly with the cranial part of the right thoracic duct, which, growing cranialward along the aorta, has crossed the midline together with this blood vessel (Fig. 20). On the right side the situation is somewhat different, probably as a result of the progressive reduction and final disappearance of the right dorsal aorta. In those embroys in which, exceptionally, this dorsal aorta had not disappeared at the moment when the early thoracic duct had started to grow out cranialward, this duct may be seen to accompany this aorta, reaching the neighbourhood of the right jugular vein and sometimes coming into contact with sprouts of the same-sided lymph sac (Fig. 19). In some other cases, where a persistent right dorsal aorta is met, the right thoracic duct splites into two processes: one of which extends to the right, whereas the other reaches the left jugular area (Fig. 11). In most embryos, however, only a few small remnants of the right dorsal aorta remain and caudal sprouts of the right jugulo-axillary lymph sac either end blindly in the loose connective tissue directly lateral to the oesophagus (Fig. 18) or have fused with cranial extensions of the para- and subtracheal lymph plexuses (Figs. 19 and 20). Besides these anastomoses, connections also may have been formed with the more laterally situated internal thoracic lymph plexuses.

In the same way, lymphatics grow out from the axillary portion of the jugulo-axillary lymph sac. Their extension along the blood vessels has progressed even further, and in the loose connective tissue of the axilla the first networks

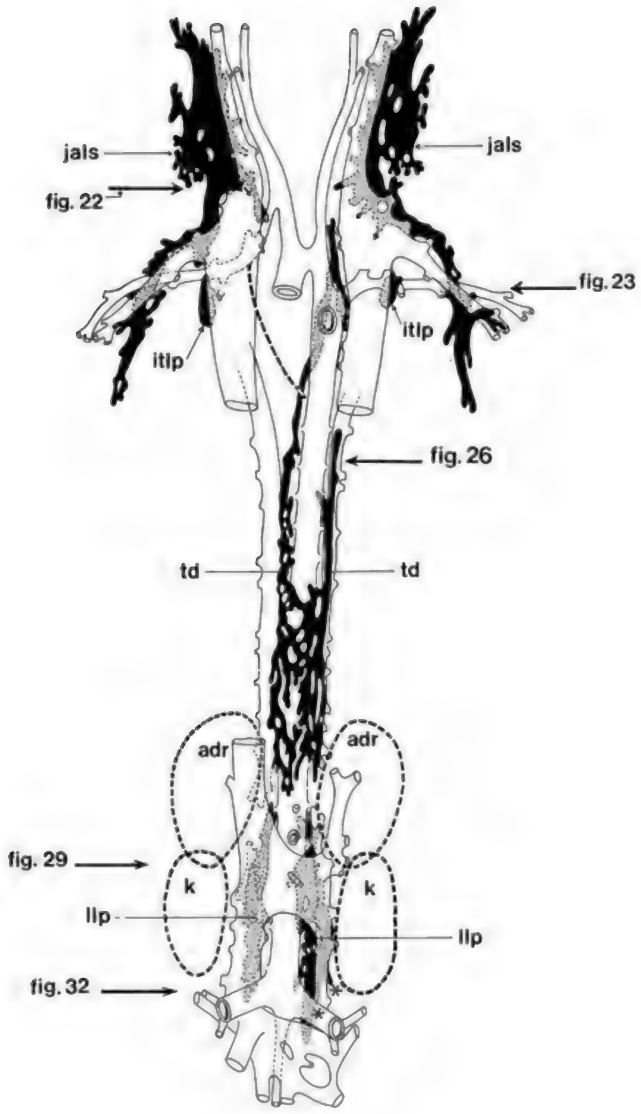

Fig. 18. × 17. Graphic reconstruction of the jugulo-axillary lymph sacs (*jals*), internal thoracic lymph plexuses (*itlp*), thoracic ducts (*td*) and lumbar lymph plexuses (*llp*) in their relation to the main prevertebral blood vessels of a 22 mm embryo, anterior view. It demonstrates the isolation of most of these primordia. Connections are but found between the left lumbar and iliac lymph plexuses (indicated by asterisks) and between both thoracic ducts

may be seen. The first mentioned lymphatics reach with the brachial artery and vein into the arm, with the scapular arteries and veins into the shoulder and with the lateral thoracic artery and veins into the lateral thoracic wall. In some embryos the lymphatics, which in the preceding period started to grow out "backward" along the subclavian vein, come into contact with the caudal part of the jugulo-axillary lymph sac, thus forming a second pathway between the jugular and axillary portions of the lymph sac.

The difference in size between both venous communications of the lymph sac, already observed in the previous phase, becomes much more marked now. Whereas the large aperture at the confluence of the external and internal jugular veins

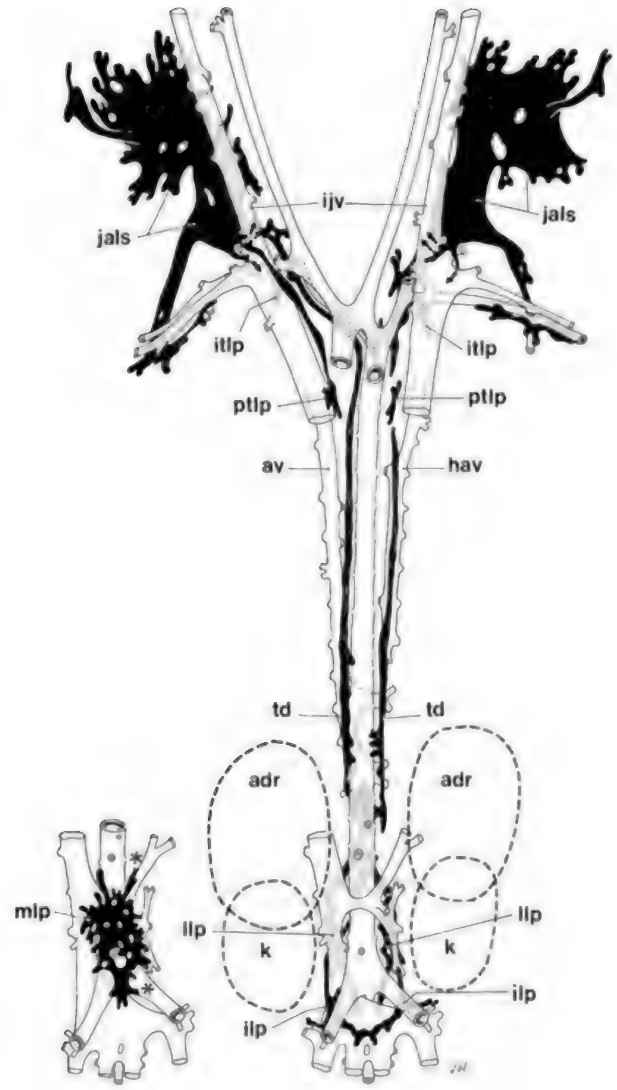

Fig. 19. × 20. Graphic reconstruction, anterior view, of the lymphatic primordia and large prevertebral blood vessels of a 22 mm embryo. With exception of the subtracheal (not shown) and left paratracheal lymph plexuses (*ptlp*) all primordia have fused; the connections between the mesenteric (*mlp*) and lumbar lymph plexuses (*llp*) are indicated by asterisks. Note the occurence of three lymphatico-venous communications (↓) between the jugulo-axillary lymph sacs (*jals*) and the internal jugular veins (*ijv*), the fusion of the right thoracic duct (*td*) and a dorsal caudal extension of the right jugulo-axillary lymph sac, and the establishment of the future bronchomediastinal lymph trunc by the fusion of the right paratracheal lymph plexus (*ptlp*) and a ventral caudal extension of the right jugulo-axillary lymph sac

widens up considerably and is guarded by real valves (Fig. 22), the other, which is now situated near the confluence of the subclavian and internal jugular veins stays behind in growth and real valves could not be identified with certainty. In six embryos of this category, these last mentioned openings even seem to be missing. Only the blind-ending slender process of the lymph sac that extends to the jugulo-subclavian confluence, forms an indication of the previous configuration.

In but a few embryos still other, small, apertures were found in the vicinity of the wide anastomosis at the confluence of the external and internal jugular

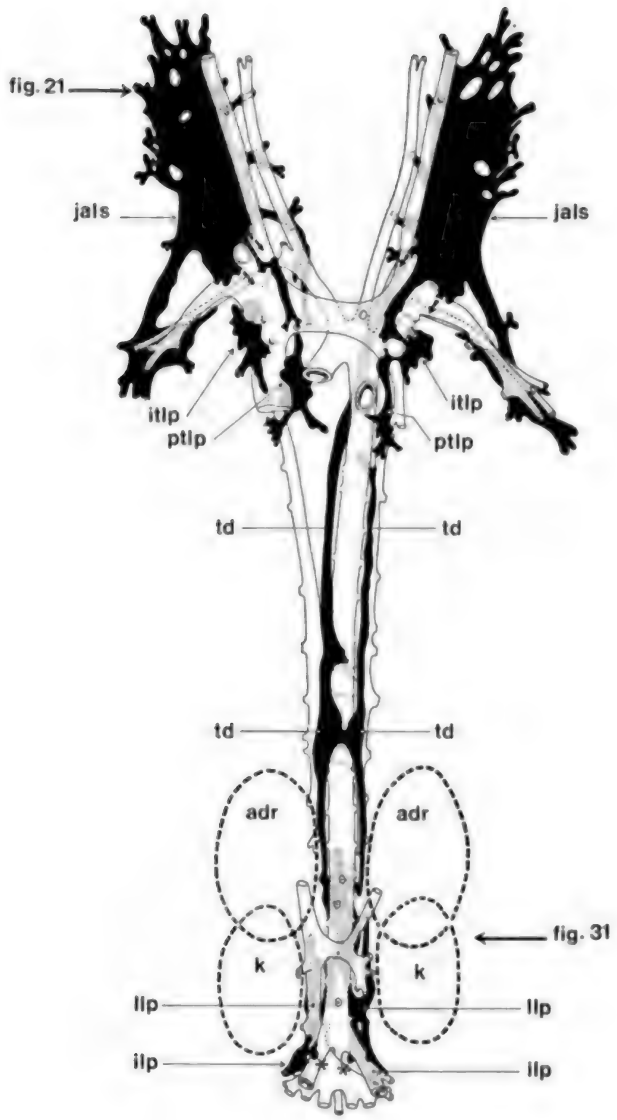

Fig. 20. × 17. Graphic reconstruction of the lymphatic system and main prevertebral blood vessels of a 23 mm embryo, anterior view. It shows the "typical" configuration of the lymphatic system after the fusion of the initially separate primordia. The right internal thoracic lymph plexuses (*itlp*) is the only primordium that is still isolated. The mesenteric lymph plexus is omitted; its form and extension is essentially similar to those of the mesenteric lymph plexus shown in Fig. 19. Those parts, which represent the initially isolated primordia may still be identified by their often sac-like appearance as compared with the slender connections between them

veins. They are situated either in the ventral wall of the internal jugular vein, often in close relation to some of the above mentioned caudal sprouts of the jugulo-axillary lymph sacs, namely those which cross the superior caval veins ventrally (Fig. 19) or in the lateral wall at a slightly more cranial level than the wide opening.

The jugulo-axillary lymph sacs are now empty in all embryos of this category. Just outside the endothelial lining of the oldest part of the lymph sac, a first arrangement of mesenchyme is visible, which probably represents the first indication of the development of other layers of the lymphatic wall.

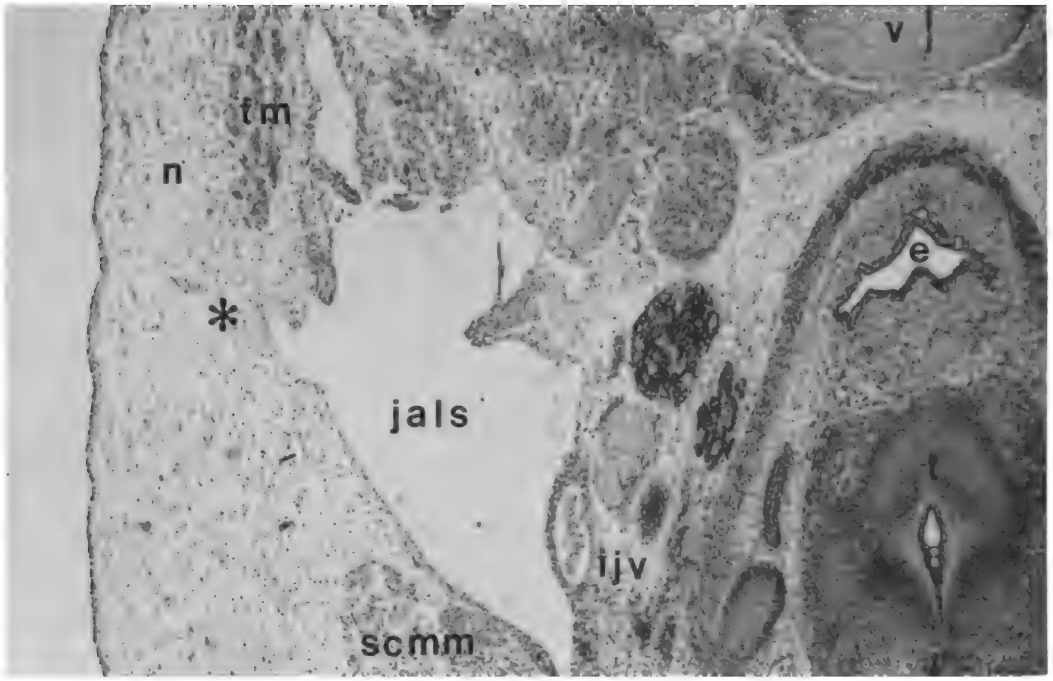

Fig. 21. × 100. Transverse section through the right jugular region of a 23 mm embryo at the level indicated in the reconstruction, shown in Fig. 20. It shows the stem of the large lateral extension (indicated by asterisk) of the jugulo-axillary lymph sac (*jals*), which passes between the trapezius (*tm*) and sternocleidomastoid muscle (*scmm*) and splits into several branches in the subcutaneous area

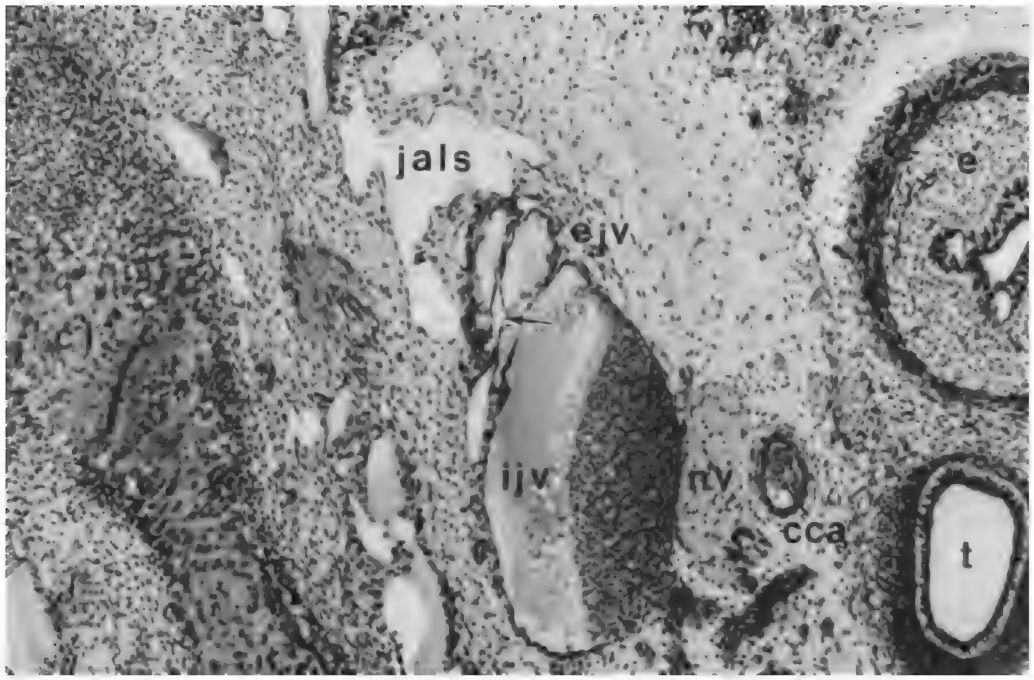

Fig. 22. × 100. Transverse section through the lymphatico-venous communication (↓) at the confluence of the external (*ejv*) and internal jugular veins (*ijv*). Note its thin valves. Level indicated in Fig. 18

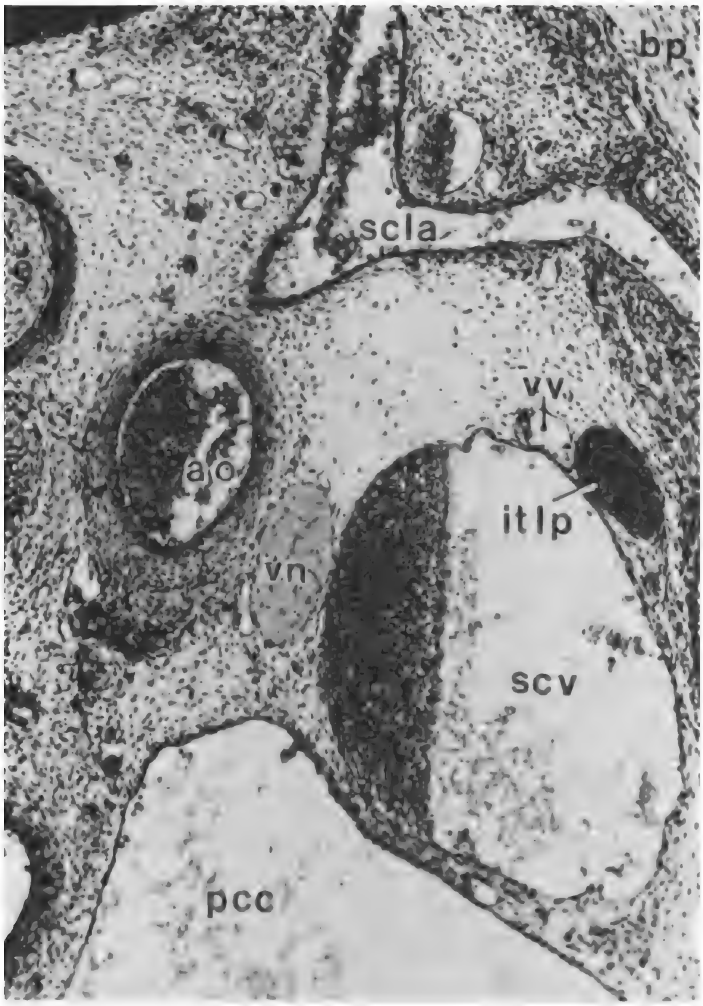

Fig. 23. × 100. Transverse section through the upper mediastinum of a 22 mm embryo at the level indicated in the reconstruction, shown in Fig. 18. It shows the left internal thoracic lymph plexus (*itlp*). Note its distinct appearance caused by the large amounts of blood cells in its lumen as compared with the almost empty neighbouring venule (*vv*)

The paired *internal thoracic lymph plexuses*, which previously were rather difficult to identify, can now be found easily. In but a minority of the observations, still isolated primordia were observed (Figs. 18 and 23). In most embryos they have fused with caudal extensions of the jugulo-axillary lymph sac and sometimes also with the paratracheal lymph plexuses of the same side (Fig. 20). In this way, several different arrangements may be encountered in one and the same embryo. When the internal thoracic lymph plexuses are still isolated, they also look as if distended by the large numbers of blood cells in their lumina (Fig. 23). After the establishment of anastomoses with the jugulo-axillary lymph sacs such contents are usually missing.

The main trunes of the *paratracheal lymph plexuses* have essentially the same extension and localisation as before, namely from the level of the ductus arteriosus to the bifurcation of the trachea (Figs. 19, 20 and 24). But, by sending out sprouts, their form has become more complex (Fig. 25) and variable anasto-

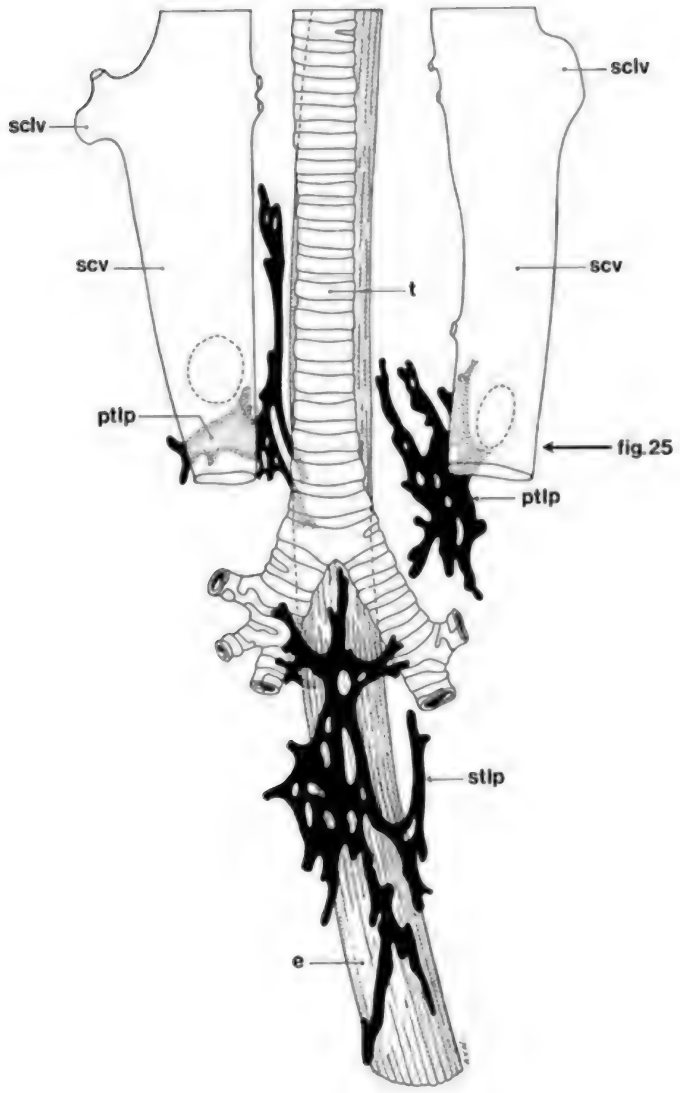

Fig. 24. × 17. Graphic reconstruction of the paratracheal (*ptlp*) and subtracheal lymph plexuses (*stlp*) and their relationships to the esophagus (*e*), trachea (*t*) and superior caval veins *scv*) of the same embryo as shown in Fig. 18

moses may have been formed with other primordia of the same side. In a cranial direction the plexuses may have come into contact with the jugulo-axillary lymph sac and internal thoracic lymph plexuses, and in a caudal direction with the subtracheal lymph plexus (Figs. 19 and 20). In but a few embryos of this category primordia are as yet completely isolated (Figs. 19 and 24). In some other embryos both plexuses have fused with the subtracheal lymph plexus without any connection with the other primordia.

Meanwhile, the *subtracheal lymph plexus* has greatly increased in size. It still extends from the bifurcation of the trachea to a level somewhat cranial to the diaphragm (Fig. 24). In addition to the connections with the paratracheal lymph plexuses mentioned, other connections were observed, namely directly with the right jugulo-axillary lymph sac and in some embryos with the right thoracic duct.

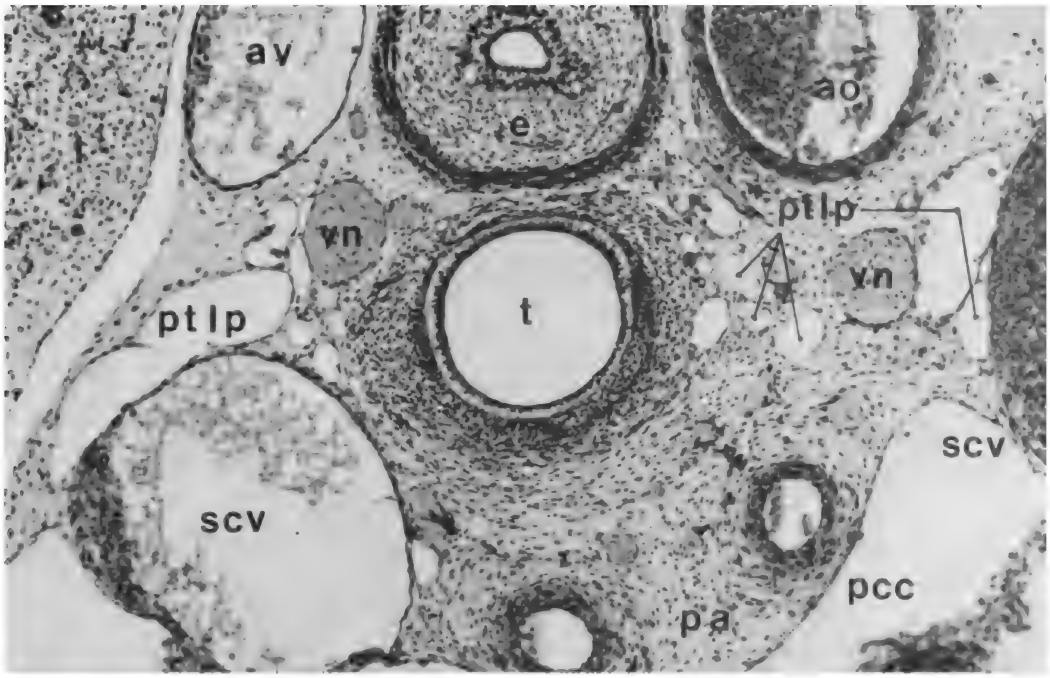

Fig. 25. × 100. Transverse section through the mediastinum of the same embryo as shown in the previous figures. It shows both complex paratracheal lymph plexuses (*ptlp*). Level indicated in the reconstruction, shown in Fig. 24

Sprouts both from the subtracheal and paratracheal lymph plexuses have now started to grow out along the bronchi and blood vessels into both lungs. In all but one embryo this process is still confined to the hilar area. In this one embryo subpleural lymphatics were also observed. It is indeed remarkable that in these young embryos a first indication of the future regional distribution of lymphatics in the lungs is found. For, while sprouts of the paratracheal lymph plexuses grow out into the upper parts of the lungs, sprouts of the subtracheal lymph plexus spread over the rest of the lungs. In this phase, the paratracheal and subtracheal lymph plexuses contain but a few blood cells.

In embryos of this category the *thoracic ducts* differ greatly in size and extension: from small and almost indiscernable vessels in one 20 mm embryo and somewhat larger, more elongated but still isolated structures in two other embryos to real thoracic ducts in the other embryos of this category (Figs. 18, 19 and 20). Although we see in the latter a very complex picture as a result of the development of variable number and combinations of anastomoses with other primordia, the thoracic ducts can still be identified as essentially elongated and rather large vessels lying closely to the ventral side of the azygos and hemiazygos veins (Fig. 26). Generally speaking, they are uncomplicated and but slightly plexiform structures, except in the lower thoracic region, where size and complexity are greatest and no differences could be observed between the right and the left duct (Fig. 27 E). From this level, more slender ducts continue cranialward also lying, initially, close to the azygos and hemiazygos veins. On the left side the ducts ends blindly at the level where the hemiazygos vein bends ventralward just before its outlet in the left superior caval vein (Fig. 27 b). On the

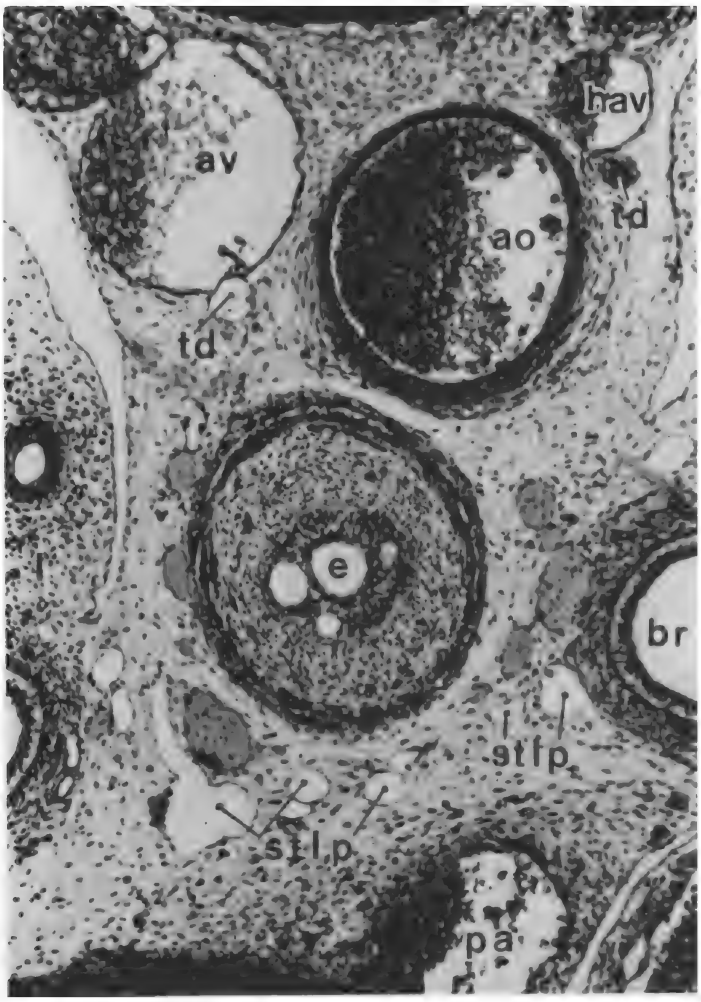

Fig. 26. × 100. Transverse section through the mediastinum caudal to the bifurcation of the trachea of the same embryo as shown in the previous figures, to show both thoracic ducts (*td*) and the subtracheal lymph plexus (*stlp*). Note the difference in content between the left and right thoracic duct. Level indicated in Fig. 18

right side, however, the duct continues further. It leaves the azygos vein shortly before it pours its blood into the right superior caval vein, and by following the descending aorta turns up at the left side of the embryo in the area to the left of the oesophagus (Fig. 27C).

Here, cranial extensions of the duct either end blindly in the mesenchyme dorsal to the aortic arch or have fused with caudal extensions of the jugular portion of the left jugulo-axillary lymph sac, which encircle the proximal parts of the left subclavian and vertebral arteries (Fig. 27A and B). As referred to above, in one embryo a very interesting variation is encountered (Fig. 19). Probably as a result of the persistence of a small right dorsal aorta, the right thoracic duct retained its position on the right of the aorta, thus parallels the persistent dorsal aorta, crosses the oesophagus dorsally and ends in a lymph plexus around the proximal parts of the right subclavian and vertebral arteries, which is quite similar to the situation usually found on the left side. In this respect yet another interesting variation was observed. Although occurring in an embryo

of the former group, it has to be mentioned here because of its rather far developed thoracic ducts. In this 19 mm embryo the right thoracic duct divides into two processes: one paralleling a persistent dorsal aorta and communicating with the jugulo-axillary lymph sac of the same side and the other paralleling the descending aorta and communicating with the jugulo-axillary lymph sac of the other side (Fig. 11).

At the level of the diaphragm anastomoses have been formed between the thoracic ducts and the lumbar lymph plexuses (Figs. 19 and 20). In but two younger embryos these have not even started to develop. In all other embryos a marked discrepancy was observed in the grade of development between those of the right and of the left side. For, whereas in all embryos communications between the right thoracic duct and one or both lumbar lymph plexuses have been established, in a large number of embryos similar connections between the left thoracic duct and the lumbar lymph plexuses are either completely absent or have a distinctly smaller diameter than those on the right side. Furthermore it was interesting to note that in accordance with this observation the left thoracic duct may contain large numbers of blood cells, which is in sharp contrast with the (almost) emptiness of the right thoracic duct (Fig. 26). But perhaps even more remarkable is the fact that, when small anastomoses between the left thoracic duct and the lumbar lymph plexuses exist, the most cranial part of the blind-ending duct is often distended by blood cells, while the lower portion caudalward gradually becomes lesser filled with blood as if evacuation is just taking place.

The *mesenteric lymph plexus* increases greatly during this period (Figs. 19, 28, 29 and 31). Its largest, sac-like portion is always found near the proximal part of the left renal vein (earlier: the intersubcardinal anastomosis). Long, slender and mostly plexiform processes extend in a cranial direction or along the inferior caval vein and the left suprarenal vein or, passing between the autonomic nerve plexuses in the hilar regions of the adrenal glands and the aorta, meet cranial extensions of the lumbar lymph plexuses. Caudally, the mesenteric lymph plexus extends to the bifurcation of the aorta or, as observed more often, yet further caudally as far as the area ventral to the caudal (sacral) veins. Passing cranially to the common iliac arteries, they may join caudal sprouts of the lumbar lymph plexuses here, or passing caudally to these arteries they may meet medial sprouts of the iliac lymph plexuses. Finally, lateral sprouts of the plexus have maintained their position as far as the mesonephric hili. Only in the youngest embryos of this group could still isolated mesenteric lymph plexuses be identified (Fig. 28).

Although occupying the same area as before, namely dorso-lateral to the abdominal aorta, the *lumbar lymph plexuses* have become much more complex structures now (Figs. 18, 19, 20, 29, 30 and 31). Of these two plexuses, the left one is markedly more extensive than the right one. Possibly, this phenomenon has something to do with the regression of the cranial part of the left sacrocardinal and caudal part of the left subcardino-sacrocardinal anastomosis. In but one embryo a completely isolated right lumbar lymph plexus is found (Fig. 18). In all other embryos connections have been established with other primordia. Thus, summarizing, the following ways of coming into contact were observed: between both lumbar lymph plexuses themselves at different levels (Figs. 19, 20 and 31); just beneath the diaphragm between the lumbar lymph plexuses and the mesenteric lymph plexus (Fig. 29) and between the lumbar lymph plexuses and the

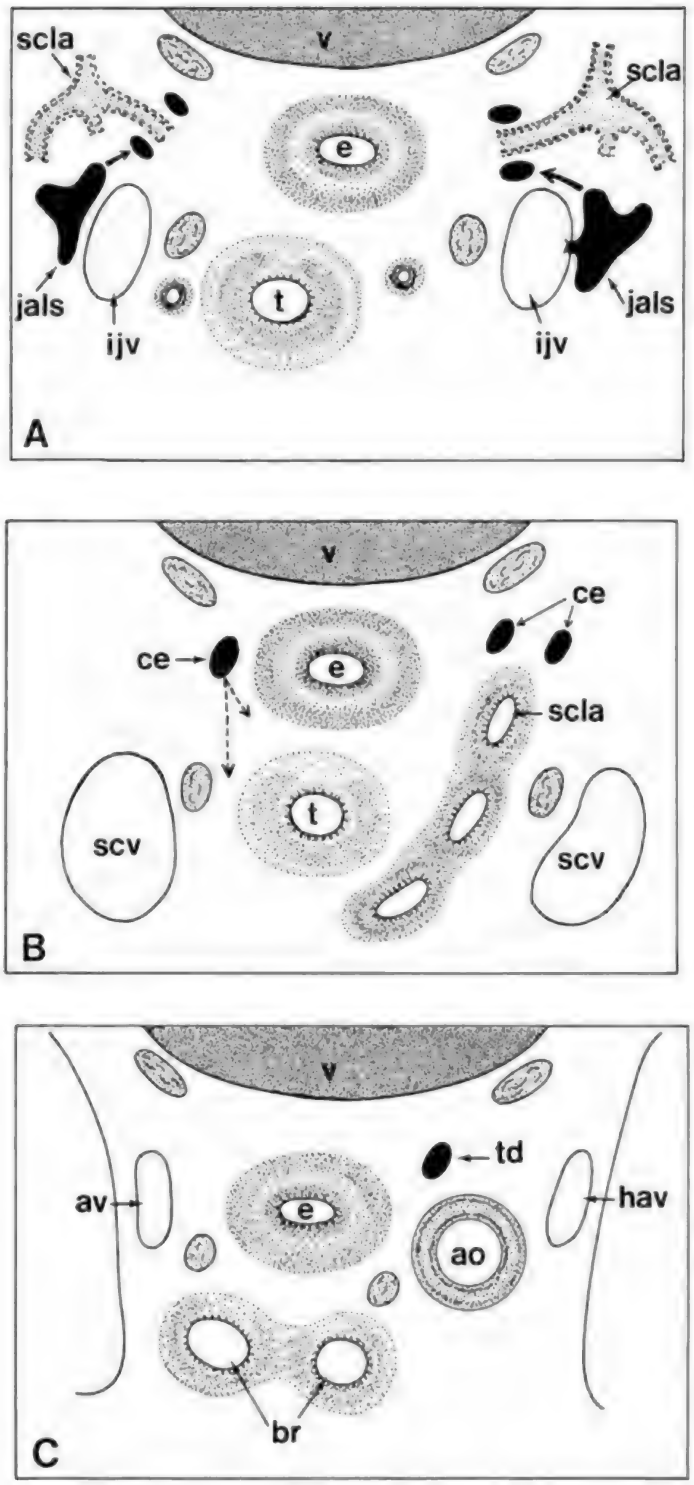

Fig. 27 A—C

Fig. 27. Diagrammatic transverse sections at different levels of an embryo of about 23 mm to illustrate the way by which the asymmetry in course of the thoracic ducts is effected. For explanation see text

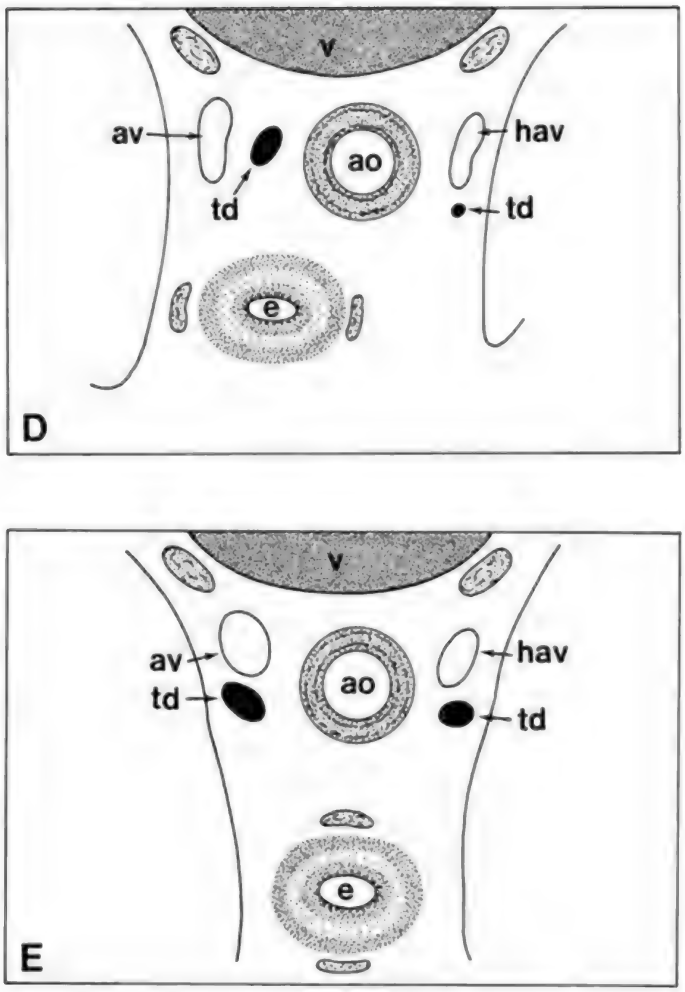

Fig. 27 D and E

thoracic ducts (Figs. 19 and 20); around the iliac blood vessels between the lumbar lymph plexuses and the iliac lymph plexuses (Figs. 18, 19, 20 and 28) and, again, between the lumbar lymph plexuses and the mesenteric lymph plexus (Fig. 20).

In all embryos of this phase, *iliac lymph plexuses* were easily identified. In but a few embryos these are still isolated (Figs. 28 and 32). In the other embryos communications have been formed with the lumbar and mesenteric lymph plexuses as described before (Figs. 19, 20 and 28). The only evidence of their separate origin is a sudden and great increase in diameter of the lymphatics near the iliac vessels. Sprouts parallel the internal and external iliac veins over a short distance. In embryos, in which no connections with other abdominal lymph plexuses and the thoracic ducts proved to exist, the primordia are partially or completely filled with blood cells (Fig. 32).

E. Fifth Phase (Embryos from 25 to 33 mm C-R Length)

The lymphatic system of the younger embryos of this category is essentially similar to that of the oldest ones of the former group. Indeed, all previously

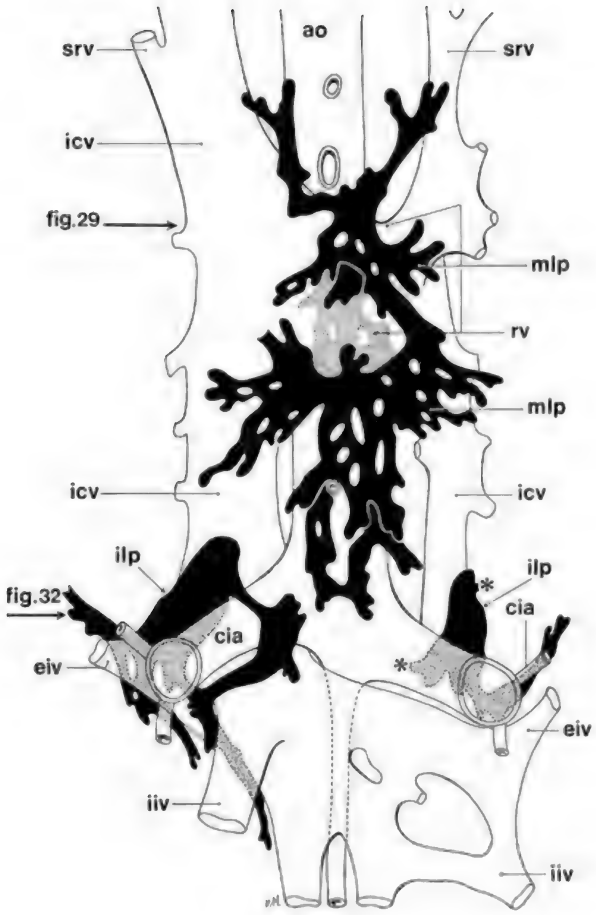

Fig. 28. × 17. Graphic reconstruction, anterior view, of the mesenteric (*mlp*) and iliac lymph plexuses (*ilp*) and their relationships to the aorta (*ao*), common iliac arteries (*cia*), inferior caval veins (*icv*) and left renal vein (*rv*) of the embryos, the reconstructions of which are shown in the Figs. 18 and 24. The connections between the left lumbar lymph plexus and iliac lymph plexus are indicated by asterisks

isolated primordia have fused and in all cases one continuous system is found. The sprouts already described in the previous group have grown out somewhat further and some new ones arose.

In contrast, in the older embryos an extensive outgrowth of lymph vessels from the main trunes into almost all directions has started. Mostly, they follow blood vessels, which results in a regional distribution of lymphatics. Thus, from the jugular portion of the jugulo-axillary lymph sac (after this phase named: jugular lymph trunes) extensive plexuses have developed into the head and neck regions. While from the small connections between this part and the axillary part small vessels arise toward the suprascapular area, considerable lymph trunes extend from the axillary portion (later named the axillary lymph trunes) to the subscapular area, the loose connective tissue of the axilla, the arm and the lateral thoracic wall. The jugular and axillary lymphatics join up near the permanent lymphatico-venous communication at the confluence of the external and internal jugular veins, which is clearly situated now in the cranial angle of the jugulo-subclavian confluence. Here, yet other lymph trunes empty into the sac. They arise caudally and are, specified from ventral to dorsal: the substernally lying internal

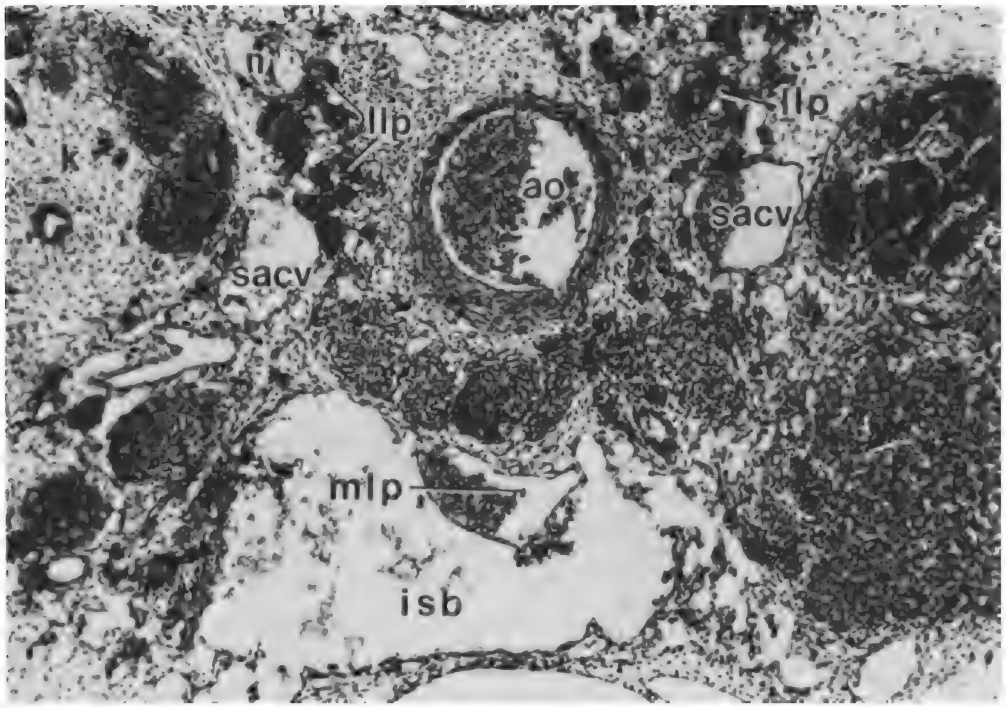

Fig. 29. × 100. Transverse section through the abdomen of a 22 mm embryo at the level indicated in the reconstructions, shown in the Figs. 18 and 28. It shows the topographical relationships of the lumbar (*llp*) and mesenteric lymph plexuses (*mlp*) to the other retroperitoneal structures

thoracic lymph plexuses (later named: internal thoracic lymph truncs), which accompany the arteries and veins of the same name and give rise to lymph plexuses expanding into the median part of the ventral thoracic wall; the sub- and paratracheal lymph plexuses (later named: bronchomediastinal lymph truncs), which are situated more dorsally, near the trachea and oesophagus and form extensive plexuses in both lungs, heart and rest of the mediastinum; most dorsal the thoracic ducts, which are lying just ventral to the azygos and hemiazygos veins and near the descending aorta, which form the link between the abdominal and jugular lymphatics and give rise to more or less regularly occurring segmental branches into the dorsal and lateral thoracic wall. The asymmetrical configuration of the thoracic ducts, previously described, does not change.

At the level of the diaphragm, both thoracic ducts continue caudalward and pass into an irregular plexus dorsal to the abdominal aorta. In this plexus, both lumbar and mesenteric lymph plexuses (later named: the para-aortic and intestinal lymph truncs respectively) empty. From the lumbar lymph plexuses, lymphatics originate, which run mainly to the dorsal abdominal wall, diaphragm, adrenal glands and kidneys, while from the mesenteric lymph plexus lymphatics grow out mainly toward the intraperitoneal organs and the gonads. These lumbar and mesenteric lymph plexuses in turn continue into the large lymph plexuses around the iliac arteries and veins, which come from the former iliac lymphatic primordia (Fig. 33). From these plexuses (later named: iliac lymph truncs) lymphatics have grown out into the sacral and gluteal regions, legs, ventral abdominal wall and intrapelvic organs.

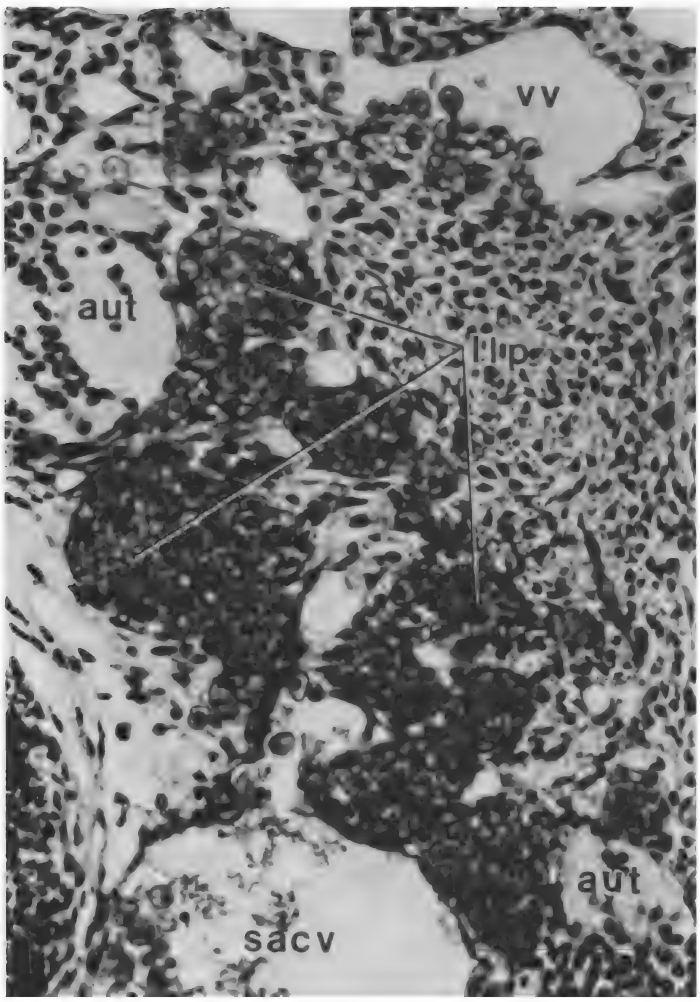

Fig. 30. × 250. Detail of the right lumbar lymph plexus (*llp*) as shown in the previous figure. Note the complexity and constrast in contents of this plexus as compared with the venous system (*vv*)

The lymphatic system holds but a few blood cells. Valves are as yet completely absent. Just outside the wall of the oldest parts of this system, mesenchymal condensations mark the position of the first lymphoid tissue, which probably has started to develop now.

Surveying the whole of the lymphatic system at the end of this stage, one gets the impression that the principal plan of the lymphatic system is ready now and that no more essential changes as to the main trunes will occur.

IV. Discussion

Concerning the methods employed in this investigation into the development of the lymphatic system, it was already stated that the single morphological criteria, used in the identification of lymphatics, were mostly not decisive, especially in the younger embryos. Thus the question arises if these criteria lead

Fig. 31. × 100. Transverse section through the abdomen of a 23 mm embryo at the level indicated in the reconstruction, shown in Fig. 20. The mesenteric lymph plexus (*mlp*) shows its largest dimension. The lumbar lymph plexuses (*llp*) are interconnected by some anastomoses at this level. Note the complete absence of blood cells in the primordia; compare with Figs. 29 and 30

to reliable interpretations of the observations. As was stated before, they mostly proved to be of variable value. The most important criterion to differentiate veins from lymphatics was based upon the fact that venous networks communicate with the large veins by wide openings, whereas connections between the lymphatic plexuses and the veins either seem to be completely absent, or are very few in number, in which cases they occur at some special sites and have a very characteristic, split-like, form. In addition, more peripherally too, no connections with the venous plexuses are established in spite of the fact that both structures are practically interwoven. Evidently, it is rather difficult to ascertain such behaviour of both types of vessels, although it proved to be possible by following them in complete series of slides. Fortunately, other features like differences in form, contents and topographical relationship, and the possibility to compare these criteria in embryos of succeeding phases of development, mostly provided sufficient support.

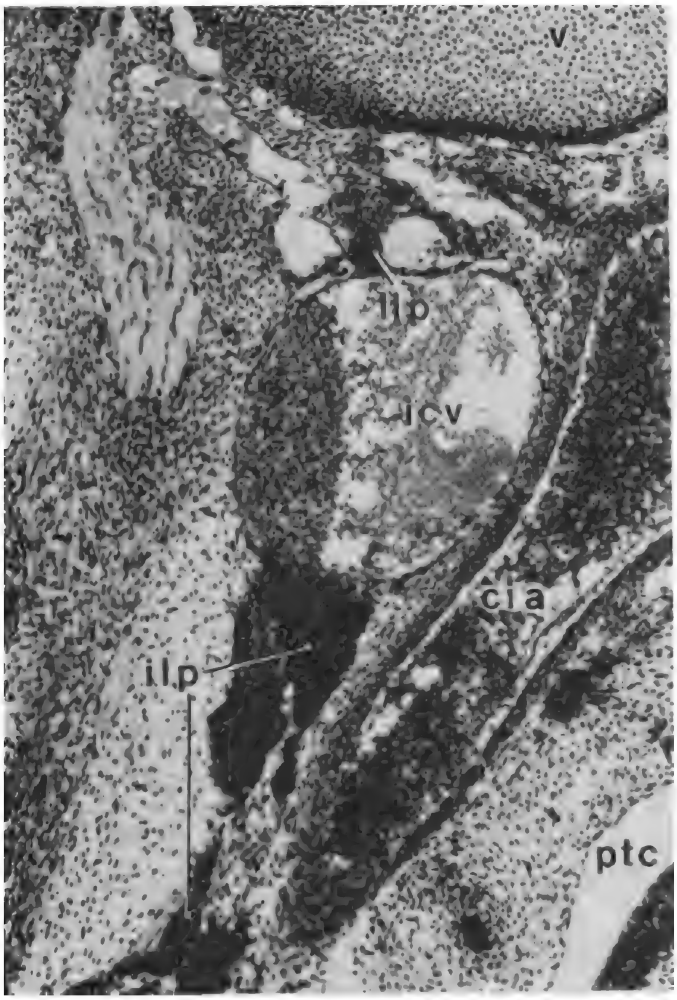

Fig. 32. × 100. Transverse section through the pelvic region of a 22 mm embryo of the level indicated in the reconstructions, shown in the Figs. 18 and 28. It shows the right iliac lymph plexus (*ilp*) and the most caudal extension of the right lumbar lymph plexus (*llp*)

In almost all previous investigations, the early development and, above all, the origin of the lymphatic system held a central position. Although from the material studied now, in which relatively few very young embryos were available because of the scarcity of young embryos sufficiently well preserved for the study of this very vulnerable system, no definite conclusions as to the origin of the lymphatic system could be drawn, strong evidence supports the opinion that the lymphatics sprout from primitive embryonic veins. For, continuity between veins and lymphatic primordia was observed repeatedly, and, in those cases in which this continuity could not be ascertained, the primordia seemed from the very beginning to be lined by flat cells that did not seem to differ from those lining neighbouring veins. Nevertheless, one has to conclude that in spite of this morphological similarity both endothelia are different almost from the very beginning. This is demonstrated both by the special transition between the two, namely their peculiar split-like communication, and by the observation that except for these early connections none other will develop later. On the other hand, it proved

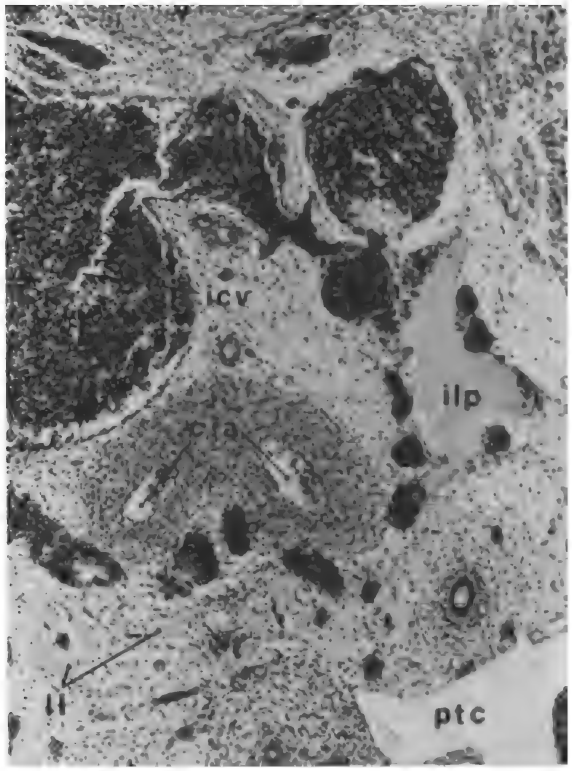

Fig. 33. × 100. Transverse section through the pelvic region of a 33 mm embryo to show the left iliac lymph plexus (*ilp*). In contrast to the veins (*vv*), no blood cells are observed in its lumen

impossible to confirm the observations by Kampmeier (1931, 1960, 1969) about the occurrence of extra-intimal spaces as an essential stage in the development of the lymphatic system in man. As Sabin (1913), E. R. Clark (1940), a.o. did before, we may suspect that real artefacts are involved. This opinion is based on the following considerations:

1. If these (perivenous) spaces in the mesenchyme should represent as essential part in the development of the lymphatic system, as Kampmeier stated, one may expect that such pictures, described and shown by him in each of the embryos studied by him, and rather fewer in number than the material studied now, could be observed without dificulties in this investigation. Similar pictures, however, were seen but very rarely and only, as will be explained below, under special circumstances.

2. For, in those cases, in which such pictures indeed were actually observed, it was clear from other observations (e.g. the loosening of blood vascular endothelium and some kinds of epithelium) that these pictures in all likehood had to be considered as artefacts caused by some autolysis or unsuccessful fixation of the embryo.

3. In those very exceptional cases in which the endothelium of blood vessels proved to be intact and similar pictures, as demonstrated by Kampmeier, where met, it was sometimes possible to follow these "shrunken remnants of veins in regression" as Kampmeier interpreted them, and to prove continuity between this

endothelium and the lining of structures unmistakebly lymphatic of nature, as for example the jugulo-axillary lymph sacs.

4. Moreover, identical pictures were met in embryos which were much older than those in which, according to Kampmeier, lymphatics were formed by coalescence of extra-intimal spaces. By form, position, contents, etc. it became clear that lymphatics, too, were involved.

Considering the last points (3 and 4), it seems conceivable now to suggest that one deals with differences between the blood vascular and lymphatic endothelium as to their resistance to post-mortem changes and fixatives. Whether the cause of this phenomenon has to be sought in differences between blood and lymphatic endothelium or in the number of "anchoring filaments", as was demonstrated by Leak a.o. (1966, 1968) in the tadpole, has still to be investigated.

5. Finally, it is difficult to understand the meaning of the existence of such processes as described by Kampmeier in 14 to 20 mm embryos in respect of the jugulo-axillary lymph sacs, and in 20 to 30 mm embroys in respect of the thoracic ducts and the cisterna chyli. For, from this investigation it became clear that during the first mentioned period (embryos from 14 to 20 mm) already large jugulo-axillary lymph sacs can be found and, as to the thoracic ducts and cisterna chyli, here also in the indicated period complete or almost complete structures are encountered, which, in most cases, are unmistakebly continuous with other true lymphatics. It is even possible to identify large lymphatic structures even before the moment, when the first indication in the form of perivascular spaces as demonstrated by Kampmeier may be observed. These observations are also in accordance with those of Sabin (1913), E. R. Clark (1915), Orts Llorca (1936) and Balankura (1951), who found fully developed thoracic ducts in embryos as small as 20 to 25 mm. From all this it seems most probable that, when Kampmeier spoke about "venolymphatics", because of their position and because of the stage of development, both compared with the observations from this investigation, early lymphatics are actually concerned.

Evidently these observations strongly support the ideas of Sabin, E. L. and E. R. Clark, Hoyer, a.o. about the origin of the lymphatic system. However important differences, too, have to be mentioned. Firstly, because a considerably greater number of lymphatic primordia proved to arise, and secondly, because these made their appearance much earlier in embryonic development than, particularly, Sabin believed. The last observation especially made it now possible to detect that the lymphatic primordia arise within a more limited period and in rather a specific area, namely in relation to, and in the neighbourhood of the anterior and posterior cardinal veins. This in turn calls for the conclusion that in all cases an essentially similar proces is involved, which starts cranially and continues in a caudalward direction, in accordance with the usual course of embryonic development. The findings of Sabin and Kampmeier, who placed the development of the jugular and abdominal lymphatics, for example, in rather different phases, seem almost incompatible with such a course.

In literature only Thyng (1914), describing a 17.8 mm embryo, mentioned the occurence of a rather large sac-like primordium (mesenteric lymph sac) before it would even arise, according to Sabin.

As mentioned above, the other observation, which differed considerably from those of Sabin, is related to the number of separate primordia that arise.

Although they outnumbered those observed by Sabin by far, yet no support could be given to the opinion of Lewis (1905), who thought that the main lymphatics arise from chains of fusing venules, which become isolated from the large venous channels.

Indeed it was Hoyer (1912, 1934) who suggested, but did not succeed in proving, that more lymphatic primordia develop than Sabin assumed. In his laboratory, Sabin's assistants E. L. and E. R. Clark (1920), brought new evidence for this theory by their experiments on chick embryos. But they also could not give precise information about place and time of origin.

Finally, one may wonder from where the blood cells come, which often fill the lymphatics in such large numbers for a short time. Although it was beyond the scope of this study, a brief investigation was set up, which indicated that these cells originate from two sources: both from mitotic activity of the blood cells lying in the lumen or in the wall of the lymphatics, and from the venous system reaching the lymphatics by small lymphatico-venous communications. They accumulate until connections with the other lymphatic primordia, and via these with the venous system, have been established and a rapid evacuation can take place.

Summarizing, it is evident that the lymphatic system is formed by fusion of a number of seperate primordia. These arise as outgrowths from the primitive main venous channels, acquiring almost immediately their own lymphatic character. It became evident also that in this process no role is played by (perivenous) mesenchymal spaces. The primordia arise in development near the anterior and posterior cardinal veins at 16 sites at least: seven bilaterally symmetric and two in the median plane of the embryo. They were found to arise in the subsequent regions: 1. in the jugular area: the jugular and axillary lymph sacs: 2. in the lower jugular and upper thoracic area: the internal and thoracic, paratracheal and subtracheal lymph plexuses; 3. in the lower thoracic area: the thoracic ducts; 4. in the abdominal region the mesenteric, lumbar and iliac lymph plexuses. The fact of their occurrence, the time of origin and the early development will be considered and compared hereafter with data obtained from the relevant literature. For briefness' sake, data concerning the development of the lymphatic system in animals will be mentioned only as far as mammals are concerned, and even then but very summarily.

As was described in the pervious chapter, the *jugular* and *axillary lymph sacs* were first observed in 10 to 11 mm embryos as small blind-ending vascular structures, which were very close to the lateral wall of the anterior cardinal veins, communicating with these by small and split-like apertures. While the jugular lymph sac is situated near the young external jugular vein, the axillary lymph sac can be identified near the proximal part of the primitive ulnar vein. After a short period, both sacs fuse and in 13 to 14 mm embryos only one *jugulo-axillary lymph sac* is found, which rapidly enlarges considerably.

Whereas the time of origin of these jugular lymphatics compares well with the observations by Sabin (1909, 1913), she failed to notice that this "jugular lymph sac" was formed by fusion of two or perhaps more seperate lymphatic primordia. Nevertheless, one of her reconstructions (Fig. 4, 1909) well demonstrates the stage when the jugular and axillary lymph sacs are still connected by a small anastomosis.

In accordance with her idea of the development of only one jugular primordium, Sabin described but one venous communication. By position it is the same, which is described here as situated between the jugular lymph sac and the anterior cardinal vein at the confluence of this vein and the external jugular vein.

After studying four human embryos of 10, 11.5, 16 and 22.8 mm, Lewis (1909), unlike Sabin, was of the opinion that the "jugular lymph sac" developed after fusion of four seperate and isolated venous tributuaries of the anterior cardinal vein. When compared, two of these appear to have the same position as the now identified jugular and axillary lymph sacs respectively, whereas a third primordium was situated more cranially than both of them. The possibility that more primordia arise in this area was also suggested in the present investigation by the presence of more venous communications cranial to the outlet of the external jugular vein with their anastomosis situated here. The fourth separate primordium described by Lewis as lying near the proximal part of the subclavian vein could not be traced. Lewis did not succeed in finding lymphatico-venous communications in any embryo studied. Still, he left the possibility that he did overlook them. And indeed, his pupil Thyng (1914), describing the lymphatic system in a 17.8 mm human embryo, observed wide anastomoses on both sides of the embryo at the confluence of the external and internal jugular veins and another pair of small apertures somewhat more cranially. Although Thyng demonstrated the small and slender caudal processes of the jugular part of the sac, which extend to the jugulo-subclavian confluence, he did not mention the presence of small venous communications in their tips, as we observed now.

Unlike these data from Sabin, Lewis and Thyng, which essentially compare well with our own observations, the observations and conclusions of Kampmeier (1931, 1960, 1969) differ greatly in many respects. Kampmeier was of the opinion that the "jugular lymph sacs" did not arise before the embryos have reached a length of 14 to 20 mm, when numerous vacuoles in the mesenchyme just outside some special dorso-lateral branches of the anterior cardinal veins—the so-called "venolymphatics"—have become confluent. These spaces in the mesenchyme, which are at first completely isolated, enlarge considerably until eventually in 30 mm embryos, communications are formed in the jugulo-subclavian confluence.

As stated before, no observations were made now, that would support such a view. On the contrary, from the present investigation it seems very probable that these "venolymphatics" themselves are true, although early, lymphatics. But even if one neglects this point and the allied question about the occurrence of lymphatico-venous communications in embryos up to 20 mm length, it is not easy to understand why Kampmeier in the older embryos from 20 to 30 mm, in which his observations about form and development of the jugular lymph sacs concur with those of this investigation, did not succeed in finding any communication with the veins. Perhaps this may be explained by the assumption that he looked for these communications where they are to be found in the adult, namely at the jugulo-subclavian confluence, as indicated in Fig. 1 of his article of 1931. As we demonstrated, the aperture, which indeed existed here initially (the communication between the axillary lymph sac and the vein), was very difficult to identify in embryos more than 20 mm in length. It is interesting to note, however, that during the same period the external jugular vein gradually changes its outlet—and with it that of the jugulo-axillary lymph sac—to the jugulo-

subclavian confluence, all as a result of differences in growth. This process has ended in embryos of 30 mm C-R length, when indeed Kampmeier found, for the first time, a lymphatico-venous communication in its final position.

In this context it seems most remarkable that in numerous kinds of animals these communications were observed both at the confluence of the external and internal jugular veins and at the confluence of the subclavian and internal jugular veins, not only in the embryonic phase (Higgins, 1926; Scharz Karsten, 1928; Lewis, 1905; Huntington and McClure, 1910; a.o.), but also in the adult animal (McClure and Silvester, 1909).

In addition to these data about the jugular lymph sacs in human embryos, more information is available from rather extensive investigations in animals. In part they represent the same differences in opinion as were summed up above, with on the one side Sabin (1902, 1904, 1913) and Lewis (1905), studying pig and rabbit respectively, and on the other side Huntington and McClure (1907, 1908, 1910) and Zimmermann (1940), studying cat and opossum respectively. Higgins (1926) and Schwarz Karsten (1928), studying mouse and cavia respectively, drew no definite conclusion as to the origin of the lymph sacs, but they both stated that strong evidence points to the correctness of the idea of origination from the venous system as supposed by Sabin a.o. It is, by the way, interesting to note that they seem to agree with Lewis in so far as the origin of the sac from multiple primordia is concerned.

Although no openings between the *internal thoracic lymph plexuses* and the anterior cardinal veins or internal thoracic veins could be ascertained, their position close to these veins, as observed in 13 and 14 mm embryos, and from comparisons with the situation as to the jugular lymphatic primordia, warrants the conclusions that they originate from these veins. In none of the publications up till now the occurrence of these separately arising primordia was mentioned. Probably they were either overlooked because of their smallness or thought to be extensions of the jugular lymph sacs or thoracic ducts. Yet, Lewis (1909) reproduced in reconstructions of 11.5 and 16 mm human embryos an isolated lymphatic primordium lying ventro-lateral to the most caudal part of the right anterior cardinal vein. This primordium, which was situated near the outlet of small veins, which by position must be regarded as the internal thoracic veins, was not named by him nor indicated in the text. The fact that Lewis observed this primordium in an embryo as small as 11.5 mm total length, strengthens for that matter our own idea that this internal thoracic lymph plexus arises very shortly after or perhaps even simultaneously with the jugular and axillary lymph sacs.

Nor were the *paratracheal lymph plexuses*, which were found for the first time in 12 mm embryos near the outlet of the tracheo-bronchial veins into the anterior cardinal veins, ever before regarded as separate entities. Sabin (1909, 1913), who indeed observed lymph truncs extending into both lungs in 20 mm embryos, assumed that these had grown out of the jugular lymph sacs in the same way that the cranial part of the thoracic duct did so. In embryos of the rabbit and cat, Lewis (1905) and Huntington (1910a, 1910c) respectively described structures which compare well with these paratracheal lymph plexuses, but without giving them a separate origin and own name.

The *subtracheal lymph plexus*, too, is described here for the first time. However, but few and somewhat irreliable data about this primordium were actually collected. It was the only primordium that left some doubt as to its separate

origin. Mostly, because it proved to be very difficult to exclude the presence of small connections between this plexus and the nearby paratracheal lymph plexuses with certainty. If, indeed, a separate primordium is formed, some kind of relationship to the tracheo-bronchial veins seems probable, although the possibility of outgrowth out of the pulmonary veins must be borne in mind.

Until now great difficulties have been encountered as to the origin and development of the *thoracic ducts* both in human and in animal embryos. By using injection techniques complete ducts were found in some embryos, whereas in other embryos of the same length no ducts could be demonstrated at all (Sabin, 1913). On the other hand, in studying histologically prepared material (from cat and pig embryos) often doubt arose about spaces in the mesenchyme, that were quite similar to newly developing, early lymphatics (McClure, 1908; Kampmeier, 1912).

Only because of the large number of embryos now available in a critical phase of the development of the thoracic ducts and partly showing distinctly isolated vascular structures marked either by a sac-like form or by a lumen almost distended by blood cells, it has now become possible to come to the conclusion that the thoracic ducts are formed by fusion and extension of a few separate primordia. These were observed for the first time in embryos as small as 16 mm C-R length, close to the posterior cardinal veins and somewhat cranial to the place where the supracardinal veins split off. From the occurrence of lymphatico-venous communications identified in this region in a 19 mm embryo, evidence was obtained that in all probability they originate from this part of the posterior cardinal veins (Fig. 11). From the lower thoracic region they grow out in a cranial and caudal direction. Cranially, the right thoracic duct crosses the midline by following the descending aorta, to communicate finally with the left jugulo-axillary lymph sac, whereas the left thoracic duct halts at the level of the outlet of the hemiazygos vein in the left superior caval vein. It is easy to understand now, how this interposition of separate, but otherwise almost finished, thoracic ducts may cause such a sudden appearance of complete ducts as observed by Sabin by injection of the jugular and axiallary lymph sacs.

Because in some embryos pictures were found that were highly suggestive of the existence of some more separate primordia (1–3) more cranially along the course of the future ducts, the possibility remains that some "accessory" primordia take part in the development of the thoracic ducts. Judging from their position they originate in all probability from other parts of the posterior cardinal veins.

The origin of the thoracic ducts was studied before by Sabin (1909, 1913) and Kampmeier (1931, 1969). And again their opinions conflicted. Sabin thought that the right thoracic duct had been formed by fusion of a caudal extension of the left "jugular lymph sac" and a cranial extension of the "posterior (iliac) lymph sac". She based her opinion on observations in a 21 mm embryo, in which the (right) thoracic duct extended from the upper part of the abdomen up to the level of the bifurcation of the trachea, where it ended blindly. Here it approached caudal sprouts of the left "jugular lymph sac" very closely. It is clear that this configuration compares well with what we now found in some embryos, in which the thoracic ducts caudally have fused with the lumbar lymph plexuses, whereas cranially connections with the left jugulo-axillary lymph sac still have not been established. Kampmeier, on the contrary, was of the opinion that the thoracic ducts are formed by fusion

and expansion of numerous spaces in the mesenchyme just outside the wall of regressing and isolated dorso-medial branches of the superior caval veins and ventro-medial branches of the azygos and hemiazygos veins. The thus formed channels will get their own endothelial lining and join cranially the "jugular lymph sacs" of the corresponding side and caudally the cisterna chyli, which originated in quite the same way. This initially bilateral symmetric system is changed afterwards by the development of a crossing channel between the cranial part of both thoracic ducts. This channel itself is also formed by fusion of extra-intimal spaces around a regressing venous connection, which, according to him, develops between the left superior caval and azygos veins. After this the most cranial part of the right thoracic ducts disappears and the lymph flow is directed through this crossing channel to the left "jugular lymph sac".

As stated above more generally, in this investigation, also with regard to the development of the thoracic ducts, no extra-intimal spaces in the mesenchyme, representing an essential phase in the origin of these ducts, were observed. On the contrary, even before they would arise in such a way as suggested by Kampmeier, complete or almost complete ducts are often found. Nor for that matter could the existence of the just-mentioned regressing crossing channel ever be affirmed.

Comparing all this with data from investigations in animal embryos, it appeared that Kampmeier's observations in human embryos are quite similar to those in pig embroys (Kampmeier, 1912), which in turn correspond well with those by Huntington and McClure (1907) and Huntington (1910a, c) in the cat. In another publication, however, McClure (1908) agreed with Lewis (1905) and Baetjer (1908a, b) who, after studying embryos of the rabbit and the pig respectivily, were of the opinion that the thoracic ducts developed from numerous isolated branches of the azygos vein. Lewis, indeed, demonstrated in one of his figures (Fig. 4, 1905) a slender isolated vessel at thoracic level.

The mode of origin and asymmetrical extension of the thoracic ducts as demonstrated now, may give an explanation of the numerous variations met in the adult (Davis, 1905; Adachi, 1953). As was suggested previously by Szawlowski (1888), Sabin (1913) and Orts Llorca (1936), in some of these variations, viz. when a right-sided thoracic duct outlet was concerned, a possible persistence of the right dorsal aorta might play an important role. This idea is strongly supported now by some observations in embryos, in which a persistent right dorsal aorta is paralleled by a continuation of the right thoracic duct. As was demonstrated, this duct may end into the right jugulo-axillary lymph sac, whereas in some embryos communications with the left jugulo-axillary lymph sac may also have been established. However, these configurations were but rarely encountered in the embryos studied. It seems, after all, needless to say that the theory about the development of an asymmetrical pair of thoracic ducts from an initially symmetrical plan, as was suggested by Huntington (1907, 1914), Kampmeier (1912, 1931, 1969), a.o. as far as man is concerned, is basically wrong. In this context, yet another observation by Kampmeier (1931) has to be mentioned. For he drew attention to the fact that in older embryos the left thoracic duct may end blindly. Also because of the direction of the valves, he had to assume that the lymph flow was directed caudally to the cisterna chyli, instead of by the shortest way direct to the jugular lymphatics. This observation does not only fit in well with our own findings, but also suggests that this configuration may last for a long period and, as proved by the figures of Adachi (1953), sometimes to adult life.

As was shown, the *mesenteric lymph plexus* develops amid the venous network, which forms the beginning of the intersubcardinal anastomosis. Sometimes an indication of continuity between blood and lymph vessels was observed here, but certainty about this could not be obtained. Nevertheless, from the whole picture it seems quite possible that both these blood and lymph vessels arise together. The development of the mesenteric lymph vessels was studied by Sabin (1909–1913) and Balankura (1951). Whereas Sabin described extensive sac-like structures in exactly the same position as were observed now, Balankura confined himself to the remark that no proof was found by him about the occurrence of sac-like and isolated structures, and, on the contrary, only split-like spaces in the mesenchyme around regression veins were observed. How wrong he probably was may be demonstrated by my Fig. 31.

Although form and position of this primordium, as described now, are basically not different from those described by Sabin, the primordium again was much earlier identified than she did. She thought that these lymphatics arose in embryos longer than 20 mm C-R length, whereas it now appeared that the first, although rather inconspicuous, vessel is discernable in embryos as small as 13 mm total length. This seems to fit in far better with the observation by Thyng (1914), who found a rather large and sac-like primordium in a 17.8 mm embryo.

In animal embryos but little attention was paid to the development of the mesenteric lymph plexus. It was discovered in embryos of the rabbit (Lewis, 1902, 1905), but most thoroughly studied in embryos of the pig (Baetjer, 1908a, b; Heuer, 1908; Sabin, 1912, 1913; Reichert, 1921). Descriptions of McClure (1910) and Zimmermann (1940) studying embryos of cat and oppossum respectively, did not differ from those of other parts of the lymphatic system.

Until now the lumbar and iliac lymph plexuses were described as forming one primordium. In this investigation it became evident, however, that two different primordia were involved.

The *lumbar lymph plexuses* were identified at first in a 12 mm embryo. They were found amid venous networks lying dorso-lateral to the abdominal aorta, from which the proximal parts of the first lumbar veins are derived, while these change their outlet from the regressing posterior cardinal veins to the newly-formed inferior caval veins. Judging from their position, these lymphatic primordia seem to originate from these blood vascular plexus, where they grow out of the oldest venous channels, represented by part of the sacrocardinal and posterior cardinal veins.

The *iliac lymph plexuses* were situated near the developing proximal parts of the external iliac veins. Although they could not be identified in embryos smaller than 17 mm C-R length, the fact that in those embryos often rather large structures were observed seems to justify the conclusion that they may originate somewhat earlier, perhaps in 14 to 15 mm embryos. From this it may be assumed that these primordia originate together with the forming of the proximal parts of the external iliac veins, although—again—no definite connections with these veins could be ascertained.

Comparing these findings and conclusions with those of relevant literature, it became evident that there is some confusion in the nomenclature of these structures. Summarizing, it was Sabin (1901), who—looking in embryos of the pig for structures homologue to the posterior lymph hearts of the frog—indeed discovered a large and sac-like lymphatic primordium near the iliac veins, which

she named "posterior lymph sacs". At the same time, however, she observed still another bilateral primordium situated more cranially, dorso-lateral to the abdominal aorta. She then assumed it was the future cisterna chyli. The same nomenclature was used some years later in an article about the lymphatic system in human embryos (Sabin, 1909). But, in the same article there was a modification in naming the structure, which in Fig. 11 was named "cisterna chyli", whereas the same primordium, judged by its topographical relations, in the next reconstruction (Fig. 12) was named "posterior lymph sac", while extending from the diaphragm to the iliac veins, from which they should have sprouted. After some years, however, she again changed her mind (Sabin, 1911, 1912, 1913). She was now of the opinion that this "posterior lymph sac" did not originate from the iliac veins extending cranialward, but on the contrary continues caudalward after having developed from the renal veins. Probably because of this extension to and further outgrowth along the iliac veins they were named from now on "iliac lymph sacs". The occurrence of a separate primordium originating from the iliac veins was not mentioned again, although both Sabin and Reichert (1921) were impressed by the fact that in the pig the caudal portion of this "iliac lymph sac" often widens up very suddenly, reaching remarkably large dimensions.

It seems probable now that Sabin's problems resulted from ignorance about the development of two separate primordia. For the rest, Sabin's observations differ from the own findings only as to the time of origin of these lymphatics.

But few investigations are available about the development of these primordia in animal embryos. Except for the observations made by Sabin (1911, 1912, 1913) and Reichert (1921) in embryos of the pig, which lead to conclusions already given in essence in the previous text, only those by Lewis (1905) and Zimmermann (1940) in embryos of the rabbit and the oppossum respectively could be found. The conclusion of both are in agreement with their more general idea about the development of the lymphatic system and offer no new points of view.

The mode of development of the lymphatic system, as is demonstrated and discussed here, implies of course most interesting explanations of the rather varying forms of the (normal) lymphatic system, the occurrence of some congenital disorders and the existence of lymphatico-venous anastomoses beyond the jugular region. Although it was very tempting to compare both, it was considered better to confine the contents of this article strictly to the origin and early development of the human lymphatic system.

Postscriptum

After the completion of this article my attention was drawn to a contribution of Töndury and Kubik in the "Handbuch der allgemeinen Pathologie"[2]. In this chapter they offer a short review of the most relevant data about the development of the lymphatic system in man, supplemented with own observations and beautiful pictures. Because of the rather concise character of their descriptions I will refrain from discussing their observations in detail and prefer to give my attention to their conclusions.

In my opinion the most important result of their investigation is the fact that they actually dropped Kampmeier's theory, although it looks sometimes as

2 Zur Ontogenese des lymphatischen Systems. Handbuch der Allgemeinen Pathologie. Bd. 3, T. 6. Springer-Verlag. Berlin, Heidelberg, New York (1972).

if they are trying to make one believe the opposite (p. 22). They observed, like many others before, in the early phases of the development of the lymphatic system small vessels markedly distended by blood cells and in continuity with the large veins. According to them these vessels have to be considered as direct precursors of some parts of the definitive lymphatic system, because no signs of regressive alterations caused by the developing lymphatic channels as was claimed by Kampmeier, were found at all. By this the most essential part of Kampmeier's theory has lost its base. On the other hand, they did observe in case of the jugular lymph sacs the same phenomena as Kampmeier described, but their interpretation is quite different. In their opinion these reflect a process by which isolated endothelium-lined mesenchymal spaces enlarge by fusion with similar clefts forming just outside their endothelial lining. This lifted lining would be preserved and subsequently form the septa, which are transversing the sacs of older embryos in large numbers. These lymphatic spaces of mesenchymal origin fuse with the first mentioned vascular structures of venous origin which constitute the part of the sac near its venous outlet. They refuse to consider these pictures as the result of a process of artifical shrinking.

Besides pointing to the improbability that within a very short period from one single layer of endothelium large septa consisting of connective tissue and endothelium will be formed, some other objections can be made against their interpretation.

As is clearly visible in their Figs. 7 and 9, this process of loosening of the endothelial lining involves not only the mesenchyme-derived endothelium but also the endothelium lining those parts near the venous outlet which is of blood vascular origin, and especially the endothelium lining the side of the sac approximate to the internal jugular vein. Here in fact the whole of the endothelial lining is participating in the forming of septa, but, as is easily demonstrated in successive phases of development, no septa are formed on this side of the sac at all.

Furthermore, the occurrence of similar clefts described and shown by them around the stems of the brachial plexus of nerves proves that shringking has taken place indeed even in these well preserved embryos. For, they are often observed in young embryos indeed, but also around those nerves which apparently do not become surrounded by lymphatics during the next stages of development. Their Fig. 36, that shows lymphatic spaces completely surrounding nerves, represents a characteristic configuration that is only found around the cervical nerves 3—which according to its position to the sternocleidomastoid muscle is shown—and 4. Finally, if their interpretation is correct, then it seems rather strange that they did not mention a similar process in the development of the other lymphatic primordia, thus forcing one to accept two different modes of development: one for the jugular lymph sacs and another for the other primordia.

It may be concluded that there is substantial doubt about the correctness of the interpretation of the observations of Töndury and Kubik, which, in my opinion, are merely related to processes of artificial shrinking.

V. Summary

Previous studies resulted in controversial opinions about the origin and the early development of the lymphatic system in man. Some authors believed that

the lymphatics developed from a few venous sprouts which soon acquired a specific lymphatic character and, as a result of centrifugal growth, contacted each other and extended into the surroundings. Others were of the opinion that the lymphatics arose from spaces in the mesenchyme which confluenced in a centripetal direction and secondarily opened into the veins. Still others suggested that these two modes of development were both involved.

In order to find out which of these views was correct, the development of the lymphatic system was re-investigated in 40 human embryos ranging from 8 mm total length to 33 mm C-R length (estimated ages: 35 to 65 days). The embryos were serially sectioned at 7 or 10 microns and the sections stained with haematoxylin and azophloxin. Characteristic stages in the development of the lymphatic system were recorded in graphical reconstructions (Figs. 1, 5, 6, 10, 11, 18, 19, 20, 24 and 28).

The development of the lyphatics was proved to start in the stages from 10 to 14 mm total length. In these stages, successively the following isolated lymphatic primordia made their appearance: bilaterally: the jugular and axillar lymph sacs, the internal thoracic and paratracheal lymph plexuses, the thoracic ducts and the lumbar and iliac lymph plexuses; and singularly in the median plane: the subtracheal and the mesenteric lymph plexuses. These primordia which in fact may derive from more than one lymphatic structure, all developed in close relation to the anterior and posterior cardinal veins and their tributaries, but solely the lymphatico-venous connections in the jugular regions were permanent. The jugular and axillar lymph sacs soon enlarge considerably and fuse to form a single jugulo-axillar lymph sac. In the stages from 15 to 20 mm C-R length, this structure divides into wide jugular and axillar portions, which remain interconnected by a narrow intermediate portion. Simultaneously, the other primordia develop into isolated plexuses greatly varying in extent and complexity. Due to growth and to the formation of new sprouts, in the stages from 20 to 25 mm C-R length the various lymphatic structures come into contact with one another. Caudal extensions of the jugulo-axillary lymph sac anastomose with the internal thoracic, paratracheal and subtracheal plexuses and the right thoracic duct, the left one ending blindly cranially. Caudally, the two thoracic ducts come into contact with the cranial parts of the lumbar plexuses which may develop into the cisterna chyli. From the caudal parts of these plexuses anastomotic vessels reach the mesenteric and iliac plexuses. The number, length and course of these anastomoses show marked individual variations. In embryos exceeding 25 mm C-R length, many sprouts, extending into peripheral regions, develop from the continuous central main lymphatics.

The observations show that in man the lymphatic system arises in the form of seven double and two single isolated primordia which are most probably of venous origin. The subsequent development of the lymphatics is solely due to centrifugal growth and sprouting.

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